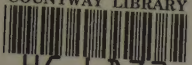


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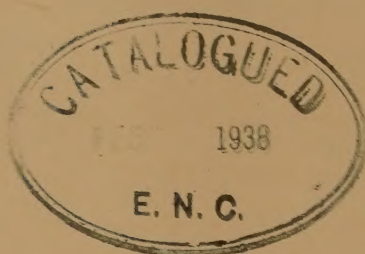
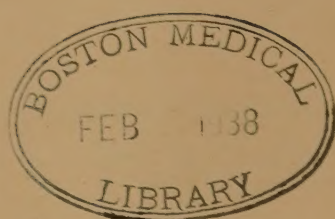
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NEW BOOKS AND PUBLICATIONS

APES, MEN AND MORONS, by Earnest Albert Hooton. 1937. Size, $6 \times 8\frac{1}{4}$ inches. Pages ix + 307. Index. Glossary. Cloth. Price, \$3.00. G. P. Putnam's Sons, New York.

"Bright Past and Dim Prospect of a Tottering Biped" is the title of the introductory chapter in this book. From this start, Doctor Hooton traces man's evolutionary development, considers his present status, and points out his probable future. The chapter titles give some slight indication of the witty treatment which causes readers to chuckle with amusement even while realizing the seriousness of the subject; but for adequate appreciation of its sound scientific value and its sparkling literary brilliance each person must read for himself the entire book. Part I, Monkey to Man to Moron, contains the following chapters: Apology for Man; Pride and Prejudice in Estimates of Man's Antiquity; The Age of Man; New Discoveries and Disputes Concerning Primate Progress; Fresh Views of Prehuman Problems; Teeth through Time; The Enigma of Fossil Man; Biology and Fossil Man. In Part II, The Biology of Human Races, the subjects are: Prologue to Anthropological Indiscretions upon Race and Nationality; The Biology of Primitive Human Societies; Plain Statements about Race; Aboriginal Racial Types in America; What is an American; and Relation of Physical Anthropology to Cultural Anthropology. Part III, Humanity Halts: The Need of Evolutionary Guidance, offers four chapters: The Eugenics Bogy; An Anthropologist looks at Medicine; Man as Director of Human Evolution; and What Must We Do to be Saved?

CONGENITAL ABSENCE OF ONE-HALF OF THE SCROTUM IN A DOG

C. W. HOOKER, J. M. DOUGLAS AND R. D. KORNEGAY

Departments of Medicine and Physiology, School of Medicine, Duke University

TWO FIGURES

The literature contains descriptions of scrotal anomalies of various types such as bifid scrota, total deficiency in intersexes, and suppressed development in cryptorchid individuals, with the suppression involving one or both halves of the scrotum depending upon whether the cryptorchidism is unilateral or bilateral. There is, however, apparently no record of total deficiency of one-half of the scrotum while the other half is fully developed. We have had the opportunity to study a dog which presented this anomaly.

The dog described here, an adult mongrel with a predominance of the German Shepherd strain, attracted our notice while under experiment in the physiology laboratory, and nothing is known of its previous history. Excepting the genital abnormalities to be described the animal was in every respect normal.

The scrotum (fig. 1) was somewhat lateral in position, situated to the left of the midline in a position corresponding to that of the lateral half of a normal scrotum. Palpation revealed only one testis and gave no indication of there being even a rudimentary right half of the scrotum such as may occur in unilateral cryptorchidism. The single testis completely filled the scrotal sac and distended it tautly. Careful examination revealed no raphe and no scarred area which should have been present had the condition been due to injury.

Dissection of the pelvis and abdomen disclosed a testis on the right side suspended in the abdominal cavity just cranial to the internal inguinal ring. On the left side a probe could be introduced into the internal ring and passed through the canal into the scrotum. On the right side the inguinal canal ended blindly at a distance of 1.5 cm. from the internal ring. Aside from the abdominal position and reduced size of the right testis, the internal urinogenital organs were all normal in appearance.

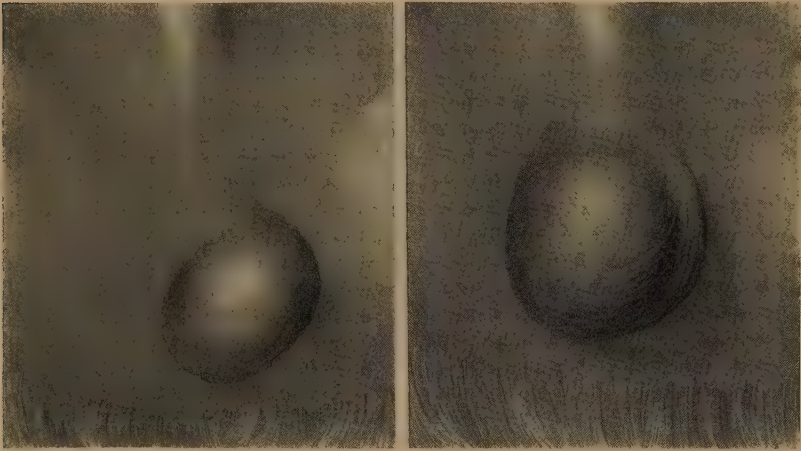


Fig. 1 Congenital absence of the right half of the scrotum in the dog here described.

Fig. 2 Rudimentary left half of the scrotum in a unilaterally cryptorchid dog.

The scrotum was found upon dissection to contain all the normal tunics, typical in their arrangement except for the relations associated with the absence of the septum.

Throughout the experiment of some 4 hours, during which the animal was anesthetized, an erection was maintained. At death, produced by chloroform, the penis returned to the non-erect state and appeared normal; there was no evidence of a partial duplication of any part nor of any incompleteness of formation. In the erect state the glans was rather larger than normal and somewhat inflamed; a yellow, viscous fluid discharged slowly from the urethral orifice.

The mode of origin of this scrotum is of some interest. Destruction of the absent portion is highly unlikely in view of the absence of scar. Atrophy attendant upon cryptorchidism or obscuring of the defective scrotum by distention of the normal side is excluded by comparison with scrota of dogs possessing but one scrotal testis. In such cases the empty half can be distinctly seen (fig. 2) and on dissection is identical in structure with the normal scrotum. The raphe is always seen. The obvious explanation is the formation in the embryo of but one scrotal evagination. In the absence of the other fold there is formed an external coat on the median side rather than a septum, just as in cases of bifid scrota (Howden, 1898; Brites, '29; deLima, '30) in which each half is comparable to the present specimen in relation of its tunics.

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A CASE OF MECKEL'S DIVERTICULUM WITH UNUSUAL VASCULAR RELATIONSHIPS

VINCENT DERBES AND MARLIN B. HOGE

Department of Gross Anatomy, Tulane Medical School

TWO FIGURES

Beginning with Meckel's classic description of the diverticulum ilei in 1812 a voluminous literature relating to this condition has been reported; the most recent comprehensive review being that of Schaetz ('25). The case herein presented is of interest because of its somewhat exceptional size and especially because no similar vascular arrangement, i.e., connections of omphalo-mesenteric (vitelline) artery with the inferior epigastric, ileal and obliterated hypogastric artery, has been previously described. The specimen was discovered in a dissecting room cadaver—a white male, aged 27, who had died of pulmonary tuberculosis. No symptoms referable to the diverticulum had been noted in the hospital records of this case.

DESCRIPTION

Measuring 22 cm. in length, the diverticulum arises from the ileum 76 cm. from the iliocecal junction. The diameter, which is uniform until the tip is reached, is 2.6 cm., only slightly larger than that of the ileum (2.5 cm. (fig. 1). At the point of junction with the ileum there is a slight constriction on the side toward the jejunum. The terminal portion tapers abruptly, as shown in the same figure, into a non-patent cord-like structure anchored to the umbilicus. There is no mesentery. The diverticulum is twisted three quarters of a turn, the twist being gradual and involving its whole length.

In figure 2 the patent omphalo-mesenteric vessels may be seen running along the 'mesenteric' border of the diverticulum. At the parietal end of the diverticulum these are joined by large branches from the right inferior epigastric vessels. Usually, according to Hudson and Koplik ('32), the omphalo-mesenteric vessels are reduced to a partially canalized fibrous

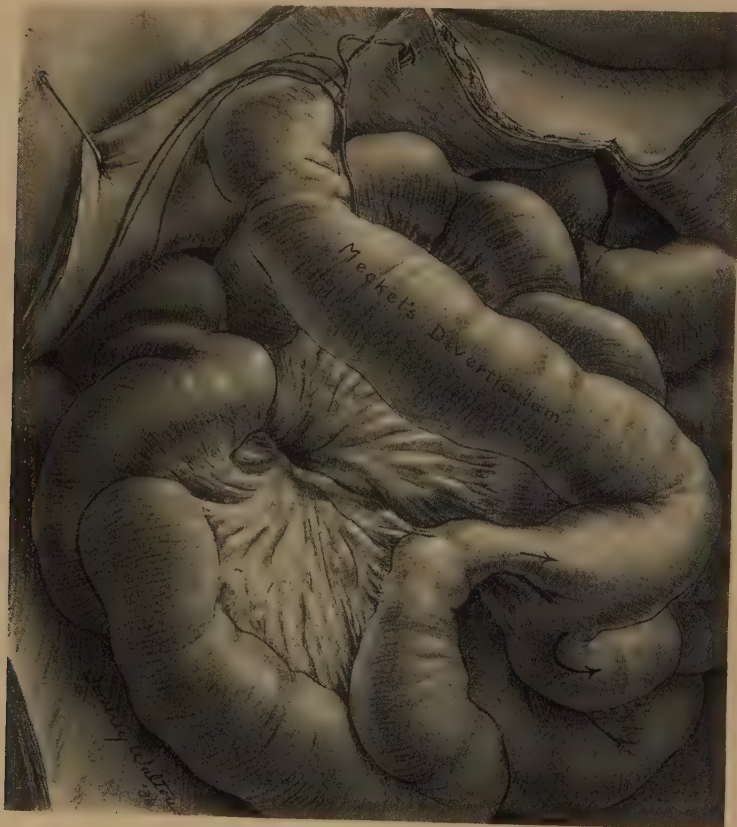


Figure 1

cord. From this omphalo-mesenteric inferior epigastric anastomosis there runs an impervious arterial connection to the right obliterated hypogastric (umbilical) artery. In addition two arteries (both non-patent) run inferiorly to be lost in the fascia surrounding the urachus (allantois). No connection to the ligamentum teres hepatis (left umbilical vein)

is discernible. On the visceral end the omphalo-mesenteric vessels join the ileal branches of the superior mesenteric vessels.

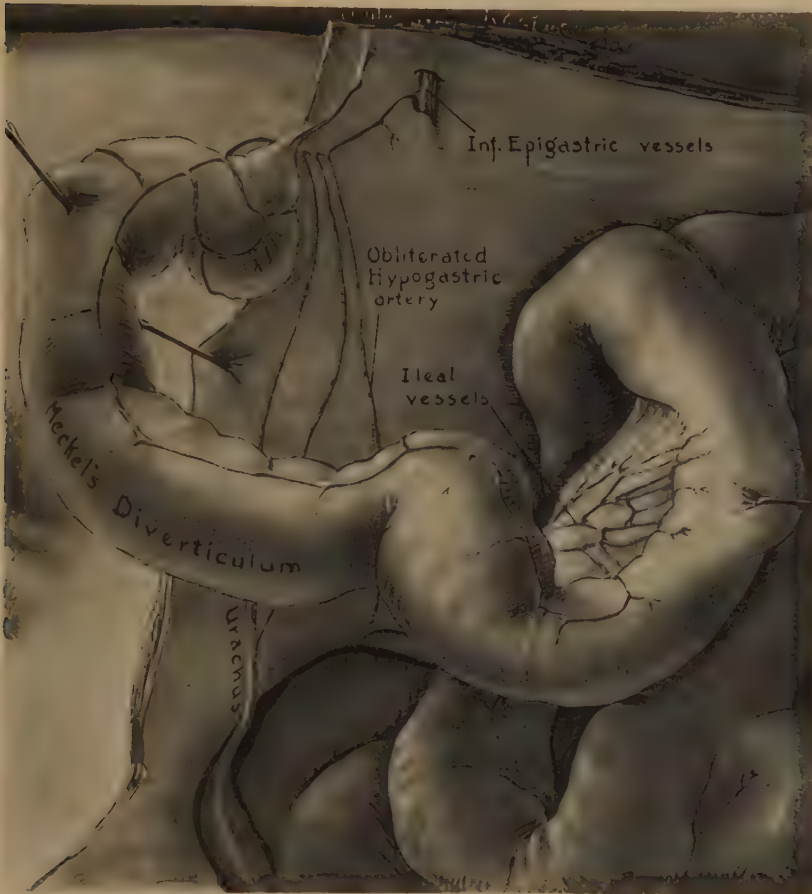


Figure 2

Sections were made in such a manner as to give a representative picture of the microscopic anatomy. In general the histology is that of the ileum. Plicae circulares are only faintly suggested and are widely spaced. The mucosa resembles that of the ileum but no Paneth cells are distinguishable; this may be due to unsatisfactory fixation. One

solitary lymph nodule is seen but no Peyer's patches are observed. There is a moderate number of lymphocytes scattered in the mucosa and submucosa. The submucosa, muscularis and serosa present nothing of note. No areas resembling gastric mucosa and no islands of pancreatic tissue are seen. The appendage (tip of vitelline duct) is fibromuscular in character and highly vascular. There is no lumen and vestiges of epithelial lining (cell nest, etc.) are lacking.

DISCUSSION

The size of this diverticulum, though somewhat uncommon, is not entirely unique; Moll ('26) having reported one 73 cm. in length and McMurrich and Tisdall ('28) one 114 cm. According to Sabin ('16), Evans ('12), and others, the omphalo-mesenteric artery is mainly a continuation of the thirteenth ventral segmental artery but also receives reinforcement from the ninth to twelfth. The ventral segmental arteries from the fifteenth somite caudally form a plexus of capillaries which invests the corresponding caudal portion of the alimentary canal and the stalk of the allantois. This plexus gives rise to the paired allantoic arteries. A few capillaries extend dorsalward from the allantoic arteries just at the point where these arteries pass ventral to the coelum. These capillaries grow lateral to the coelum and will eventually form the umbilical arteries in the somatopleure.

It is thus evident that embryonic connections exist between the umbilical arteries and the omphalo-mesenteric and between the latter and the plexus surrounding the allantois. It is therefore logical to view the impervious connections in this specimen as persistences of once functional vascular pathways. Although a developmental connection between the omphalo-mesenteric vessels and the inferior epigastric has not been found described, it is reasonable to suppose that in those instances in which the tip of the Meckel's is attached to the anterior abdominal wall in the region of the umbilicus, such an anastomosis could easily come about.

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QUANTITATIVE STUDIES OF THE RATE OF REMOVAL OF UREA BY LIVING BLOOD CAPILLARIES FROM EXTRAVASCULAR SOLUTIONS IN TRANSPARENT MOAT CHAMBERS INTRODUCED INTO THE RABBIT'S EAR ^{1, 2}

RICHARD G. ABELL

Laboratory of Anatomy, University of Pennsylvania School of Medicine

THREE FIGURES

It was suggested by Roux (1895) that new capillary sprouts are sent out as a direct response on the part of the endothelial cell to the passage through it of increased amounts of substances. This hypothesis was further developed by Clark ('18), and according to it the endothelium reacts by sending out a new sprout, which eventually becomes a new capillary, when the amount of substance passing through the endothelial wall exceeds a certain quantity. Precise information concerning the rate of passage of known amounts of chemical substances through capillary endothelium under conditions which make possible direct observation of sprout formation is, however, lacking. Consequently the aforementioned hypothesis has not been tested experimentally.

It was felt that if the rate of passage of a known, innocuous chemical substance through capillary endothelium could be increased experimentally while the blood capillaries into which this substance was passing were being observed directly with the microscope, information concerning the relation between

¹ This work was aided by a grant to the department of anatomy at the University of Pennsylvania by the Rockefeller Foundation, under which the author held a fellowship.

² Brief reference to some of the results reported in this paper was made by the author (Abell, '37) at the 1937 meeting of the American Association of Anatomists.

the passage of this substance through the endothelium and the formation of new blood capillary sprouts might be secured.

For a series of experiments of this sort, a chamber was needed into which a known volume of a solution of some desired chemical substance could be introduced, and into which it could be sealed in such a way as to permit access of the solution to living blood capillaries, growing in a region in which sprout formation could be studied with the microscope.

Such a chamber is the moat chamber, described by Abell and Clark ('32) and designed to make possible microscopic studies of the reaction of living blood capillaries (also of arterioles and venules) to chemical substances sealed within an extravascular reservoir called a 'moat.' This type of chamber was used in the experiments described in the present paper.

Urea was chosen as the chemical substance to introduce into the chambers because it diffuses readily through living tissue (Marshall and Davis, '14; Gad-Andresen, '21, and Galan and Houssay, '27), the ease with which it passes through capillary endothelium being well known.

In order to show that the reactions of the blood capillaries, and also of the arterioles and venules, in moat chambers following the introduction of urea solutions into the moat, were actually due to the urea, it was necessary first of all to determine quantitatively the rate at which the urea passed into and was removed by the vessels.

The experiments to be described in the present paper are a series of quantitative studies of the rate of removal of urea by living blood capillaries from solutions introduced extravascularly into moat chambers. The results of these experiments have made apparent the relation between the rate of removal of urea by the capillaries and 1) the concentration of urea in the solutions introduced into the chambers; 2) the absorbing capillary area; and 3) the volumes of the extravascular solutions.

The reactions of the blood capillaries, arterioles and venules to the urea solutions introduced into moat chambers will be described in a subsequent report.

MATERIAL AND METHODS

1. *Construction, installation and care of moat chambers, and growth of tissue into them.* The construction, installation, and care of moat chambers, and the growth of tissue into them is described by Abell and Clark ('32). Briefly, such a chamber is composed principally of glass and mica, is oval in shape, and contains a very thin, inclosed space called a 'bay,' which communicates at one end, termed the proximal end, with two entrance holes, and at the other end with a reservoir, or 'moat.' Urea solutions are placed in the moat, and subsequently removed from it, by means of two permanently installed access cannulae. Following introduction of the chamber into a hole cut through the ear of a rabbit, living, vascular tissue, continuous with the subcutaneous tissue of the ear, grows into the bay through the entrance holes. Since the bay, at its distal end, opens directly into the moat, urea solutions installed within the moat are free to diffuse into the bay, where they pass into the more advanced capillaries and are removed by the circulation. Because the bay is transparent (it has a glass bottom and a mica top) and very thin, the capillaries can be seen clearly with the high powers of the microscope, drawn with a Leitz 'drawing eyepiece,' and photographed (compare figs. 1 and 2).

The volumes of the moats of the chambers used in the present experiments were somewhat greater than that of the chamber originally described by Abell and Clark. This increase in volume was secured by increasing the width of the moat (at its widest point) from 4 mm. to approximately 8 mm. A photograph of a moat chamber containing a moat such as just described is shown in figure 1.

2. *Method of determination of the rate of removal of urea by the blood capillaries.* The rate of removal of urea by the blood capillaries was determined as follows. Urea solutions varying in concentration from 60 to 3000 mg. per 100 cc. were introduced into the chambers, sealed within them, and left for intervals of time which varied in length from 2 to 10 days. They were then withdrawn and analyzed quantitatively for

urea. No lymphatic vessels were present in the chambers used in these experiments, consequently there was no way for urea to leave the moats except by the blood capillaries in the chambers. The rates of removal of urea by the capillaries could thus be calculated following determinations of the differences in concentration between the urea in the solutions at the time of introduction into and removal from the moats.

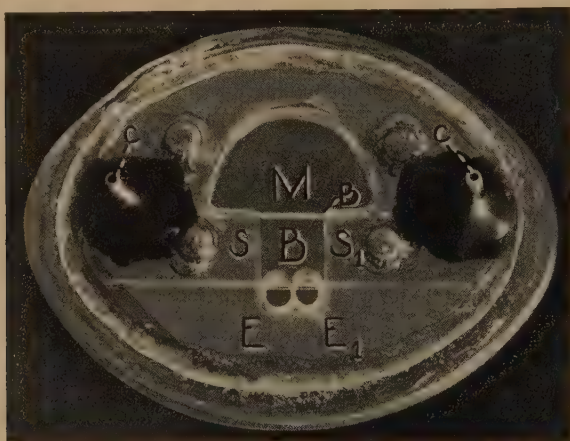


Fig. 1 Photograph of an assembled 'moat' chamber. M., moat; larger B., bay; the smaller B of the two, distal end of bay and edge of moat, also edge of one side of the bay; E and E₁, the two entrance holes; C., the access cannulae, without plugs in position, and unsealed. S and S₁ are placed over the sides of the bay. The letters E and E₁ and the smaller B of the two indicate the same structures marked by the letters E and E₁ and B on the photomicrograph given in figure 2. $\times 1.9$.

3. *Preparation and sterilization of the urea solutions.* The urea solutions were prepared by washing, with distilled water, the desired weight of urea into a 1 liter volumetric flask which contained 9 gm. of NaCl, 0.42 gm. of KCl, and 0.24 gm. of CaCl₂. Approximately 500 cc. of distilled water were added, followed by 0.15 gm. of NaHCO₃. The solution was then made up to 1 liter with distilled water. Such a solution might be termed a urea-Ringer-Locke solution, and will be designated by the abbreviation 'urea-R.-L.' solution.

Before introduction into the chambers, the urea-R.-L. solutions were sterilized by filtration through a Seitz filter.

4. *Introduction of the urea solutions into, and subsequent removal from, the moat.* The urea-R.-L. solutions were introduced into the moats with a 2-cc. Luer syringe, sterilized by dry heat. Before installation of a urea-R.-L. solution of desired concentration within the moat, the fluid content of the moat was always removed and the moat rinsed with 2 cc. or more of the solution to be introduced into it. Following rinsing, the urea-R.-L. solution was sealed within the moat and removal of urea allowed to continue as long as desired.

The urea solutions were removed from the moats with a 1.0 cc. B-D Luer syringe, and transferred directly to a Folin micro blood sugar pipette, calibrated at 0.100 cc. and 0.109 cc. From the pipette the solutions were introduced into the urea digest tubes.

No alcohol should be placed upon the access cannulae following removal from them of the sterile wax and rosin seal, since in some instances alcohol placed upon the cannulae in this way has been found to pass into the moat and to exert an effect upon the vessels in the bay. In further detail the method of installation of the urea-R.-L. solutions within the moats and of subsequent removal from them was the same as that described for solutions of methylene blue by the author ('34).

During removal of the urea-R.-L. solutions from the moats, and introduction of fresh solutions into them, the rabbits ear was fastened, by means of the protective splints, to a 4 inch $\times$ 7 inch cork-topped board held in the desired position by a ring stand clamp. During these, and all other procedures involving manipulation of the chambers, the rabbit was fastened to an adjustable rabbit board, such as described by Clark, Sandison and Hou ('31).

5. *Method of chemical analysis of the urea solutions.* The volumes of the moats of the chambers used in the present experiments were approximately 0.14 cc. and 0.19 cc., respectively, so that the method used to determine the amount of urea in the solutions removed from the chambers had to

be one that required not more than approximately 0.1 cc. of solution. The micro method described by Hindmarsh and Priestley ('24) was found to be satisfactory, and was the one used in the experiments to be described.

The urease solutions were prepared by dissolving urease tablets (Squibb's) in distilled water, a fresh solution being used for each experiment.

The NH_3 was aerated into HCl , the volume of which varied from 6 cc. to 10 cc., and in concentration from 0.05 N to 0.1 N, the volume and concentration depending upon the concentration of urea in the solution to be digested.

After nesslerization, each unknown was read against a suitable standard in a Duboseq colorimeter and the concentration of urea in each calculated.

It was found advisable not to attempt duplicate analyses of solutions removed from the moats, since in many instances the volume of solution secured was little greater than 0.10 cc. Consequently an analysis of the same volume of a control urea-R.-L. solution was made for each analysis of a urea-R.-L. solution removed from the moat.

In order to determine the relation between the rate of removal of urea from the moats, and the concentration of urea in the blood, urea analyses of the blood from two rabbits were made. The method used for these analyses was the same as that for the analyses of the urea-R.-L. solutions removed from the moats.

6. *Condition of the capillaries at the time of introduction of the urea solutions into the moats.* Records of the growth of the blood vessels into the chambers were made with a Leitz 'drawing eyepiece' during the period of vascularization of the bays preceding installation of the urea-R.-L. solutions within the moats, and photomicrographs were taken frequently throughout the pre-introductory periods. Thus the history of the vessels within the chambers was known, and the capillaries shown to be of normal appearance at the time of introduction of the first urea-R.-L. solutions into the moats. Microscopic and photomicrographic records were continued during

the periods of removal of urea by the capillaries, i.e., following introduction of the urea-R.-L. solutions into the moats.

It was shown by Abell and Clark ('32) that the removal of the fluid content of the moat, and the introduction of sterile mammalian Ringer solution in its place, produces no abnormal variation in the growth or appearance of the vessels in the bay. Consequently in the present experiments there is no reason to believe that the rate of removal of urea by way of the vessels was affected by any mechanical manipulations involved in introduction of such solutions into the moat.

OBSERVATIONS

1. Observations dealing with the relation between the concentration of urea in the solutions introduced into the chambers and the rate of removal of urea by the capillaries. Two chambers, which will be designated as chambers 1 and 2, were used in the experiments to be described. Chamber 1 was introduced into the left ear of an adult brown female rabbit, while chamber 2 was inserted in the right ear of an adult female albino rabbit. Both rabbits were in good health at the time of introduction of the chambers, and remained so throughout the experiments. Ten experiments were performed with chamber 1, and two with chamber 2. These experiments may perhaps best be described by referring to the individual chambers.

a. Chamber 1. A urea-R.-L. solution was first introduced into and sealed within the moat of this chamber when the most advanced capillaries were 1.14 mm. from the moat. The concentration of urea in milligrams per 100 cc. in the solution introduced into the moat, the length of time that the solution remained in the moat, the concentration of urea in the solution removed from the moat at the end of the experiment, as determined by chemical analysis and by calculation, and the average rate of removal of urea from the moat by the capillaries in milligrams per 100 cc. of moat content for this first experiment and the nine subsequent experiments performed with this chamber are summarized in table 1. Figure 2 is a photomicro-

graph of blood vessels in the bay and under the sides of the bay of chamber 1 taken during experiment 9.

The absorbing capillary area remained comparatively constant in all but experiments 5 and 6, in which it increased, due to growth of capillaries from the bay into the moat.

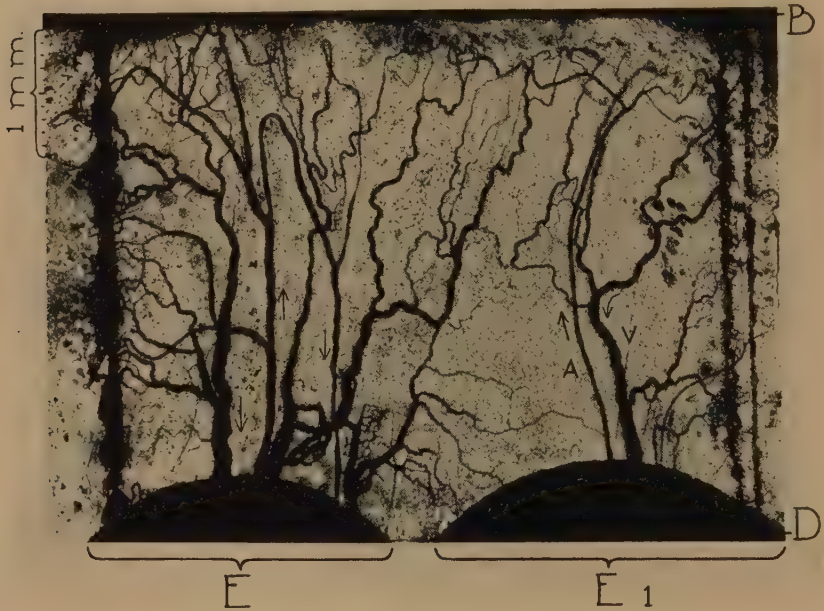


Fig. 2 Photomicrograph showing blood vessels in the bay, and also under the sides of the bay, of chamber 1. This photomicrograph was taken during experiment 9, 118 hours following the installation of a urea-R-L. solution containing 73 mg. of urea per 100 cc. within the moat. This solution was removed and analyzed quantitatively for urea $\frac{1}{2}$ hour after this photomicrograph was taken. Brackets E and E₁ are placed below the edges of the two entrance holes through which the new tissue invaded the bay. The bay extends from the line D to the line B. The line B indicates the position at which the bay communicates with the moat. The arrows pointing upward are placed next arterioles, A; those pointing downward next veins, V. $\times 22.4$.

Growth of capillaries into the moat began during experiment 5 and continued during experiment 6, this continued growth into the moat resulting in a continuously increasing absorbing capillary area during these experiments. At the beginning of experiment 7, one side of the chamber began to slip out

of the ear of the rabbit. This resulted in a mechanical tension upon the vessels which destroyed the capillary plexus in the moat, but not in the bay. Because of the fact that the capillaries in the moat were destroyed while those in the bay were not, the capillary area exposed to the urea solutions was reduced to that present before the vessels grew into the moat.

TABLE 1

Details concerning removal by blood capillaries of urea from urea-R.-L. solutions introduced into the moat of chamber 1. The ten experiments form a continuous series and were carried out in the order given. The disparity between the observed and calculated result in experiment 6 is due to the marked increase of absorbing capillary area that occurred in this experiment due to growth of capillaries into the moat. A 10-mg. blank was deducted from all results of analyses of solutions below 100 mg. per 100 cc. The recoveries from solutions of higher concentrations were usually slightly below the theoretical values as shown by analyses of control solutions.

EXPERIMENT NO.	CONCENTRATION OF UREA (MILLIGRAMS PER 100 CC.) IN THE UREA-R.-L. SOLUTIONS INTRODUCED INTO THE MOAT	HOURS THE SOLUTIONS RE- MAINED IN THE MOAT (t)	CONCENTRATION OF UREA (MILLIGRAMS PER 100 CC.) IN THE SOLUTIONS REMOVED FROM THE MOAT AT THE END OF TIME t , AS DETERMINED BY CHEMICAL ANALYSIS	CONCENTRATION OF UREA (MILLIGRAMS PER 100 CC.) IN THE SOLUTIONS REMOVED FROM THE MOAT AT THE END OF TIME t , AS DETERMINED BY CALCULATION WITH EQUA- TION 1	AVERAGE RATE OF REMOVAL OF UREA FROM THE MOAT BY THE CAPILLARIES IN MILLIGRAMS OF UREA PER 100 CC. PER 24 HOURS, AS DETERMINED FROM RESULTS SECURED BY CHEMI- CAL ANALYSIS OF THE SOLU- TIONS REMOVED FROM THE MOAT AT THE END OF TIME, t
1	90	215	53	49	4
2	200	122	111	119	17
3	200	115	125	122	17
4	60	50	53	53	3
5	60	51	48	53	5
6	200	44	130	164	37
7	200	240	77	78	12
8	200	119	108	120	18
9	73	119	55	53	3
10	250	120	149	146	20

The side of the chamber that had partially slipped out of the ear was introduced into the ear again, without injury to any of the vessels in the bay. During the remainder of this experiment and during the subsequent experiments the capillaries did not grow into the moat, the capillary area remaining once again approximately constant.

b. Chamber 2. A urea-R.-L. solution was introduced into and sealed within the moat of this chamber when the most advanced capillaries were 0.02 mm. from the moat. In this experiment and the subsequent one performed with this chamber, the concentration of urea introduced into the moat was 3000 mg. per 100 cc. Data concerning the rate of removal of urea by the capillaries in both experiments are summarized in table 2. The capillary area remained approximately constant during both experiments, but was slightly smaller in experiment 2 than in experiment 1.

TABLE 2
Details concerning removal by blood capillaries of urea from urea-R.-L. solutions introduced into the moat of chamber 2

EXPERIMENT NO.	CONCENTRATION OF UREA (MILLIGRAMS PER 100 CC.) IN THE UREA-R.-L. SOLUTIONS INTRODUCED INTO THE MOAT	HOURS THE SOLUTIONS REMAINED IN THE MOAT (t)	CONCENTRATION OF UREA (MILLIGRAMS PER 100 CC.) IN THE SOLUTIONS REMOVED FROM THE MOAT AT THE END OF TIME (t)
1	3000	95	1200
2	3000	97	1400

2. *Observations dealing with the relation between the rate of removal of urea by the capillaries and the concentration of urea in the blood.* In order to determine the relation between the rate of removal of urea by the capillaries and the concentration of urea in rabbit's blood, three sets of duplicate analyses of the blood from two rabbits were made. The results of these analyses are given in table 3.

TABLE 3
Duplicate urea analyses of blood from two rabbits

RABBIT FROM WHICH THE BLOOD WAS TAKEN	RESULT OF ANALYSIS NUMBER 1, MILLIGRAMS OF UREA PER 100 CC.	RESULT OF ANALYSIS NUMBER 2, MILLIGRAMS OF UREA PER 100 CC.
80°	31	..
76K	33	33
76K	28	29

The average of these results is 31 mg. of urea per 100 cc. of rabbit's blood, and in subsequent calculations given in this paper which involve the concentration of urea in rabbit's blood it is this figure that is used.

3. *Observations dealing with the relation between the absorbing capillary area and the rate of removal of urea by the capillaries.* a. Chamber 1. During experiment 2, four drawing ocular records of the total length of the peripheral capillary loops in this chamber were made, one on March 23rd, one on March 24th, one on March 25th and one on March 27th. From measurements of these drawings, the length of capillary contained in the peripheral row of loops on each of these days was calculated, the average of these lengths being used for the determination of the peripheral absorbing capillary area. The measurements are given in table 4.

TABLE 4

Lengths of the most advanced capillaries in the bay and under the sides of the bay of chamber 1

DATE ON WHICH DRAWING OCULAR RECORD WAS MADE	TOTAL LENGTH OF CAPILLARY IN THE MOST ADVANCED ROW OF LOOPS IN THE BAY, IN MILLIMETERS	TOTAL LENGTH OF CAPILLARY IN THE MOST ADVANCED ROW OF LOOPS UNDER THE SIDES OF THE BAY, IN MILLIMETERS
March 23	6.71	0.66
March 24	6.63	1.05
March 25	6.16	0.97
March 27	5.84	1.15
Average	6.33	0.96

In order to make possible computation of the area of the most advanced row of capillary loops in the chamber, 100 measurements of the diameter of the most advanced capillaries, at different places along their length across the bay, were made on March 23rd. The greatest diameter found at any place was 0.026 mm., the smallest 0.007 mm., the average 0.014 mm.

Reference to the increase in absorbing capillary area in experiments 5 and 6 has already been made.

b. Chamber 2. Only one drawing ocular record was made of the total length of capillary in the most advanced loops in this chamber. Calculations from this record show that the total peripheral capillary length in the bay of this chamber during the absorption experiments was 13.16 mm., and under the sides of the bay 5.34 mm. Thus the total length of

peripheral absorbing capillary in this chamber was more than twice as great as that of chamber 1.

4. *Observations dealing with the relation between the volumes of the chemical reservoirs and the rate of removal of urea by the capillaries.* Measurements of the volume of the chemical reservoir, or moat, of chamber 1, showed it to be approximately 0.14 cc. The volume of the moat of chamber 2 was found to be approximately 0.19 cc. Hence in each experiment with chamber 2, the volume of extravascular urea-R.-L. solution sealed within the chamber was 1.36 times greater than in the case of that sealed within the moat of chamber 1.

DISCUSSION

1. *The relation between the concentration of urea in the moat and the rate of removal of urea by the capillaries.* A comparison of the results of experiments which differed only in the length of time that the urea-R.-L. solutions were left within the moat, for example experiments 7 and 8 of the series with chamber 1, indicates that the rate of removal of urea by the capillaries decreases with increase in length of time that absorption continues, the volume of the solution from which the urea is passing into the vessels remaining constant, and no fresh urea being added. This is because the concentration of urea in the urea-R.-L. solutions introduced into the moat decreases with time, due to removal of urea by the capillaries. Thus the progressive decrease in rate of removal of urea with time is due to a concomitant decrease in concentration of urea in the extravascular urea-R.-L. solution in the moat.

An examination of the results of experiments 2, 3, 8, 9 and 10 of the series with chamber 1, in which the absorbing capillary area and also the lengths of time that the urea-R.-L. solutions remained within the moat were approximately the same, shows that the rate of removal of urea by capillaries having approximately equal absorbing areas from solutions of identical and constant volume increases with increase in concentration of urea in the solutions in the moat, the urea being removed at

a more rapid rate from solutions of higher concentration than from those of lower concentration. Thus, as shown by experiment 10, the average rate of removal of urea from a solution containing 250 mg. of urea per 100 cc., introduced into and left within the moat for 120 hours, was 20 mg. per 100 cc. per 24 hours, while the average rate of removal of urea from a solution containing 73 mg. per 100 cc. introduced into and left within the moat for approximately the same length of time, was only 3 mg. per 100 cc. per 24 hours. Consequently it is evident that the rate of removal of urea by the capillaries is some function of the concentration of urea in the extravascular solutions.

The factors so far mentioned which in the present experiments determined the rate of removal of urea by a capillary plexus of constant absorbing area at any given instant of time following the installation of a urea-R.-L. solution within an extravascular reservoir, or moat, are thus the concentration of urea in the solution at the time of introduction into the moat, and the length of time that the solution has remained within the moat. Both of these factors are taken into account in the following modification of a well-known diffusion equation.³

$$\frac{C_{1t} - C_2}{C_1 - C_2} = e^{-kt} \quad (1)$$

where C_1 is the concentration of urea in milligrams per 100 cc. in any urea-R.-L. solution at the time of its introduction into and installation within the moat, C_2 the concentration of urea in rabbit's blood, in milligrams per 100 cc., C_{1t} the concentration of urea, in milligrams per 100 cc., in the urea-R.-L. solution in the moat at the end of time t (expressed in hours from the beginning of the experiment), e the base of natural logarithms, and k a constant of diffusivity derived from the experimental data secured with chamber 1, and which is 0.0053.

³For the formulation of this equation and also equation 2, which is described later in this paper, the author is indebted to Mr. Ronald B. Smith, of the Westinghouse Laboratories. In the derivation of the constant of diffusivity, Mr. Smith used only the results of experiments 1, 2, 3, 4, 7, 8, 9 and 10 of the series with chamber 1. The results of experiments 5 and 6 were not used because of the increase of absorbing capillary area in these experiments.

By means of this equation it is possible to calculate the rate of removal of urea by a capillary plexus having an absorbing area of 0.35 sq.mm. (a figure which will be discussed later, and which is the absorbing capillary area in chamber 1) from a urea solution of any desired concentration introduced into and sealed within a moat of volume 0.14 cc., (the volume of the moat of chamber 1). The graph of this equation is given in figure 3, C.

Using this equation, the concentration of urea in the moat at the end of each experiment of the series with chamber 1 was calculated, and the results compared with those secured by actual analysis (table 1).

The fact that k remains constant for the experiments reported is sufficient to suggest that the passage of urea into the capillaries from extravascular solutions in moat chambers is a process of simple diffusion, the nature of the formula indicating that the rate of diffusion of urea into the capillaries is directly proportional to the concentration gradient. That the rate of diffusion of substances in solution is proportional to the concentration gradient is well known.

The distance through which the urea diffused in passing from the moat to the capillaries varied slightly in the different experiments. Thus at the beginning of experiment 1 of the series with chamber 1, the distance of the capillaries from the moat was 1.14 mm. During the interval of time between the beginning of experiment 1 and the end of experiment 2, the capillaries grew forward toward the moat, and at the beginning of experiment 3 were at the edge of the moat. Consequently during experiment 3, the distance through which the urea diffused in passing into the absorbing capillaries was only the thickness of the capillary wall. Yet no significant difference in rate of removal of urea in experiment 1 and 3 due to this difference in distance was detected (compare table 1, concentrations as determined by chemical analysis and calculation).

2. *Calculation of the absorbing capillary area.* There is reason to suppose that in the present experiments the urea

diffused into and was removed by only the more advanced capillaries in the chambers, and that not all of the capillaries in the bay or under the sides of the bay were involved. Such was found to be the case with solutions of methylene blue (Abell, '34) and phosphate salts (Abell, '35) introduced into moat chambers. That it is also evidently the case with urea solutions is shown by the position in the bay of the capillaries which showed visibly an effect of urea upon their endothelium,

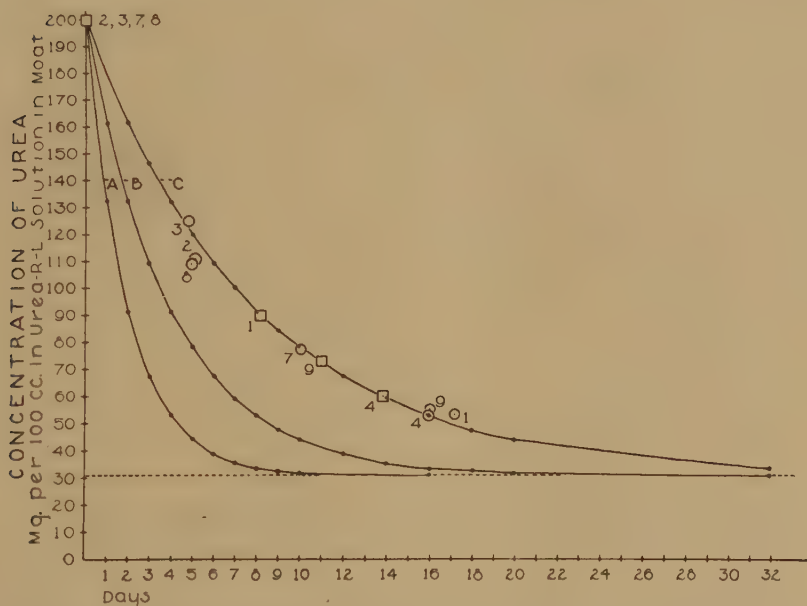


Fig. 3 Graphs showing the rate of decrease of concentration of urea in a moat of volume v_1 (0.14 cc.) produced by diffusion of urea into and removal by capillaries of absorbing areas a_1 (0.35 sq.mm.), $2 a_1$ and $4 a_1$, respectively, following the installation within the moat of a urea-R-L. solution containing 200 mg. of urea per 100 cc. Graph C, moat volume v_1 , capillary area a_1 ; graph B, moat volume v_1 , capillary area $2 a_1$; graph A, moat volume v_1 , capillary area $4 a_1$. The solid dots on graph C represent points calculated with diffusion equation (1), those on graphs A and B, with diffusion equation (2). The experimental data secured with chamber 1 are shown on graph C by the squares and circles, the numbers indicating these squares and circles corresponding to the numbers of the experiments of the series with chamber 1 (compare table 1). The squares show the positions on the graph that urea-R-L. solutions were introduced into the moat, and the circles the points at which they were removed and analyzed, and the concentration of urea found upon analysis. The dotted line indicates the concentration of urea in rabbit's blood (31 mg. of urea per 100 cc.).

following the installation of urea-R.-L. solutions within the moat.

The effect to which reference has just been made is a change in the capillary endothelium which makes it sticky toward leukocytes. The factors responsible for the development of such sticking will be discussed in detail in a subsequent report. It might be well to note at the present time, however, that sticking of leukocytes occurred when the concentration of urea in the moat was only 80 mg. per 100 cc., at which concentration the osmotic pressure of the solution in the moat was very little greater than that of the blood, and that the intensity of sticking varied with the concentration of urea in the moat. Changes in blood vascular endothelium which give rise to leukocyte sticking have recently been discussed by Clark and Clark ('35), who have shown that there are several distinct phases of leukocyte sticking and emigration which depend upon changes in endothelium, and which vary in proportion to the strength of the inciting factor.

Sticking of leukocytes to the walls of the capillaries produced by the urea-R.-L. solutions was always most pronounced in those capillary loops closest to the moat, i.e., to the source of the urea. Sticking in the capillaries just proximal to the most advanced loops, that is, those separated from the source of urea by one row of capillary loops, was always much less marked than was sticking in the capillary loops exposed directly to the solution, and sticking in the third row of capillary loops was usually slight.

Using the degree of sticking of leukocytes to the endothelium as an indication of the relative concentrations of urea at the individual capillary loops, it is evident, since the rate of removal of urea is directly proportional to the concentration gradient, that the greatest amount of urea was removed by the most advanced row of loops, that less was absorbed by the next most advanced row, a slight amount by the third most advanced row, but little or none by more proximal ones. A very rough estimate, based upon observation of sticking in the capillaries, would be that practically all of the urea was

removed by way of blood passing through the vessels of a peripheral strip of tissue which varied in width from approximately 0.5 mm. to 1.0 mm. This was true even when the concentration of urea in the solution introduced into the moat was very great, i.e., 3000 mg. per 100 cc.

When new blood vessels grow into moat chambers, they grow not only into the bay, but also for varying distances under the sides of the bay. Although the spaces under the sides of the bay are very thin, experiments with methylene blue and with phosphate buffers indicate that diffusible substances placed in the moat actually do reach the most advanced of the capillary loops present under the sides of the bay, although in much smaller amounts than reach the most advanced capillaries in the bay. In chamber 1, the depth of the spaces under the sides of the bay was 0.030 mm., while the depth of the bay was 0.100 mm. It is assumed, therefore, that 30/100 as much area was removed by way of the most advanced capillary loops under the sides of the bay, per unit of capillary area, as by the peripheral capillaries in the bay itself.

Since the most advanced capillaries under the sides of the bay remove only about 0.3 as much urea per unit of capillary area as do the most advanced loops in the bay, and since the amount of urea passing into the more proximal rows of loops decreases rapidly as the number of capillaries between them and the moat increases, it is manifestly impossible to make an exact calculation of the total capillary area involved in the removal of the urea in the present experiments which would represent an area of endothelium through each unit of which an equal amount of urea passes during an equal interval of time.

However, for purposes of comparing the absorbing capillary area of one chamber with that of another, one may assume that three-fourths of the urea is removed by way of the most advanced row of capillary loops, and the remaining one-fourth by more proximal capillaries, separated from the moat by one or more rows of capillaries. This is in accord with the observations that urea-R.-L. solutions introduced into the

moat exert a far more pronounced effect upon the endothelium of the most advanced row of capillary loops than upon that of any of the more proximal vessels, and that solutions of methylene blue and phosphate salts do not diffuse generally throughout the tissue in the bay, following their installation within the moat.

If, then, it is assumed that three-fourths of all of the urea is removed by way of the most advanced capillary loops in the chamber and one-fourth by the more proximal ones, and that the most advanced capillary loops under the sides of the bay remove 0.3 as much urea per unit of absorbing area as do the most advanced capillary loops in the bay, it is possible to compute the surface capillary area, which, if urea diffused through it and into the capillary in equal amounts in all places, would account for the removal of urea from the moat at the rate actually observed in the present experiments. Using this method of calculation, the absorbing surface capillary area involved in chamber 1 was 0.35 sq.mm., and in chamber 2, 0.81 sq.mm. The volume of the moat of chamber 1 was approximately 0.14 cc.; that of chamber 2, approximately 0.19 cc.

3. *The relation between the rate of removal of urea and the absorbing capillary area, the moat volume and the rate of decrease of concentration of urea in it due to removal of urea by the capillaries, and the moat volume and the rate of removal of urea by the capillaries.* If equation 1 is modified so as to include values for the absorbing capillary areas and moat volumes of chamber 1 and any other chamber in which the absorbing capillary area and moat volume differ from those of chamber 1 (as for example those of chamber 2), the relation between the rate of removal of urea and the absorbing capillary area, and the rate of removal of urea and the moat volume can be determined. Thus the general formula for calculation of the rate of removal of urea from a chamber having a moat volume and absorbing capillary area of any desired magnitude would be

$$\frac{C_{1t} - C_2}{C_1 - C_2} = e^{-k \frac{V_1 a_2}{V_2 a_1} t} \quad (2)$$

where, as in equation 1, C_1 is the concentration of urea in milligrams per 100 cc. in any urea-R.-L. solution at the time of its installation within the moat, C_2 the concentration of urea in rabbit's blood, in milligrams per 100 cc., C_1 , the concentration of urea, in milligrams per 100 cc., in the urea-R.-L. solution in the moat at the end of time t (expressed in hours from the beginning of the experiment), e the base of natural logarithms, k the constant of diffusivity derived from the experimental data secured with chamber 1, and which is 0.0053, and where v_1 is the volume of the moat of chamber 1, v_2 the volume of the moat of any chamber other than chamber 1, a_1 the absorbing capillary area of chamber 1, and a_2 the absorbing capillary area of any chamber other than chamber 1.

Calculation with this formula shows that a urea-R.-L. solution containing 3000 mg. of urea per 100 cc., introduced into and left within the moat of chamber 2 for 95 hours, would decrease in concentration from 3000 mg. per 100 cc. to 1300 mg. per 100 cc. Actual analysis, as shown by the results of experiment 1 with chamber 2 (compare table 2) showed that the concentration of such a solution left within the moat for 95 hours actually decreased to 1200 mg. per 100 cc. Experiment 2 with this chamber, which, as shown in table 2, was an approximate repetition of experiment 1, exhibited a decrease in concentration from 3000 mg. of urea per 100 cc. to 1400 mg. per 100 cc. The average of these results, secured by actual analysis, is 1300 mg. of urea per 100 cc., a good agreement with the calculated result. This agreement demonstrates experimentally the validity of the relations expressed in equation 2.

Equation 2, which follows from Fick's law, and is, indeed, an application of this law to the conditions of the present experiments, indicates that the rate of decrease of concentration of urea in an extravascular solution, such as contained in moat chambers, due to removal of urea by blood capillaries, is directly proportional to the absorbing capillary area (compare figure 3) and inversely proportional to the volume of the solution. It follows that the rate of removal of urea from

such an extravascular solution by blood capillaries is directly proportional to the absorbing capillary area.

Since, under the conditions of the present experiments, the rate of diffusion of urea into blood capillaries from an extravascular solution is directly proportional to the concentration gradient, and since the rate of decrease of concentration of urea in such a solution is inversely proportional to the volume of the solution, it is to be expected that during any appreciable interval of time more urea will diffuse into capillaries of the same absorbing area from a solution of larger than of smaller volume. Calculation shows that such is actually the case. Thus capillaries of absorbing area 0.35 sq.mm. will remove 0.028 mg. of urea from a moat of volume 0.14 cc. during the first 24 hours following the introduction into the moat of a urea-R.-L. solution containing 200 mg. of urea per 100 cc., while capillaries having this same absorbing area will remove slightly more urea (0.029 mg.) from a moat of volume 0.28 cc. during the same interval of time following the installation within the moat of a urea-R.-L. solution of the same concentration.

An estimate has been made of the approximate amount of urea that passes through the capillary wall, per unit of absorbing area, during the first 24 hours following the installation within chamber 1 of a urea-R.-L. solution containing 200 mg. of urea per 100 cc. Calculation shows the amount to be 0.008 mg. per 0.1 sq.mm. of absorbing capillary surface.

SUMMARY

1. A method of determining quantitatively the rate of removal of urea by living blood capillaries from extravascular solutions is described in the present paper.

2. The results obtained with this method are consistent with the values calculated by means of a formula developed from an application of Fick's law of simple diffusion. Hence the present experiments indicate that a) urea passes into blood capillaries from extravascular solutions by a process of simple diffusion; b) the rate of such diffusion is directly proportional

to the concentration gradient; and c) the rate of decrease of concentration of urea in an extravascular solution due to diffusion of urea into and removal by blood capillaries is directly proportional to the concentration gradient and to the absorbing capillary area, and inversely proportional to the volume of the solution.

This paper would be incomplete without expression of appreciation to Dr. Eliot R. Clark for proposing that the present work be undertaken, and for many valuable suggestions.

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A MODIFICATION OF MALLORY'S CONNECTIVE TISSUE STAIN WITH A DISCUSSION OF THE PRINCIPLES INVOLVED

GERMAIN CROSSMON

*Department of Anatomy, The University of Rochester, School of Medicine and
Dentistry*

Since the publication in 1900 of Mallory's anilin blue connective tissue stain, a number of modifications have been proposed. These methods, however, often yield results which are generally unsatisfactory, or else discrepant with regard to the coloration of such elements as red blood corpuscles and bone. In some cases the modifications reveal lack of comprehension of the fundamental principles of the method; in others, the difficulty is caused merely by such practical errors as over-strict adherence to a time stipulation for immersion of sections in the various reagents, failure to avoid exhaustion of the solutions, etc. Without understanding of these points, not even the original Mallory method will yield constant results.

The procedure described in this paper and explained in the accompanying notes takes account of the basic principles and results in uniformly well-stained preparations.

Mallory's original method consists in staining appropriately fixed sections in acid fuchsin, which confers a more or less uniform red color upon the tissue generally. This stain is then removed from the collagenous fibers by phosphomolybdic acid, which at the same time acts as a mordant for subsequent staining of the same fibers with anilin blue. Orange G mixed with the anilin blue, serves as a stain for myelin sheaths and red blood corpuscles. When successful, connective tissue reticulum, collagenous fibers, mucus, amyloid

and various other hyaline substances stain in different shades of blue; nuclei, cytoplasm, axis cylinders and fibrin stain red; red blood corpuscles and myelin sheaths, yellow. Cartilage, bone and dentine are blue when correctly stained.

The revised procedure is as follows:

1. *Fix in Zenker's fluid.* This fixation is essential if correct action of orange G is to be obtained. After fixation in formalin and Bouin's fluid, erythrocytes and myelin possess greater affinity for acid fuchsin and consequently are stained red.

2. *Imbed in paraffin, cut and mount as usual.*

3. *After removal of paraffin and passage through alcohols to water, place sections in modified Lugol's solution as recommended for Verhoeff's elastic tissue stain ('08).*

Iodine	2 gm.
Potassium iodide	4 gm.
Distilled water	100 cc.

Leave in this solution about 5 minutes or until microscopic examination shows complete removal of mercuric crystals. Too long immersion of sections in iodine solutions tends to undo fixation and to hinder nuclear staining.

4. *Transfer to several changes of 70% ethyl alcohol to remove all iodine from the tissue.*

5. *Wash sections in running tap water for at least 10 minutes.*

6. *Stain in Mayer's acid hemalum* (standard formula as given in Lee's Vade-Mecum). Time of staining is dependent on examination. The nuclei should be slightly overstained; nuclear differentiation is to be accomplished later by the acetic acid, a constituent of the acid fuchsin—orange G solution (step 8, below).

Weigert's iron hematoxylin may be used in preference to Mayer's acid hemalum, or the hematoxylin stain may be omitted as in the original Mallory directions. In that case acid fuchsin acts as the nuclear stain.

7. *Wash sections in running tap water for 10 minutes.*

8. *Stain in acid fuchsin—orange G mixture.*

Acid fuchsin (National Aniline Co.)	1.0 gm.
Orange G (National Aniline Co.)	0.4 gm.
Distilled water	300.0 cc.
Thymol crystals	0.2 gm.
Glacial acetic acid	3.0 cc.

This mixture keeps for months and may be used repeatedly. A suggested initial staining time is 1 minute. Sections are then transferred to distilled water (step 9) for examination. If the time has been optimum, the nuclear stain of hematoxylin will be properly differentiated and the rest of the section quite homogeneously stained red. According to Mallory's formula, orange G is a copious ingredient of his secondary stain, a mixture of orange G and anilin blue. Putting it with the acid fuchsin, as suggested here, materially lessens the amount of dyestuff needed and more consistently stains erythrocytes and myelin yellow. Failure to stain yellow is usually due to too short an immersion time in the acid fuchsin—orange G mixture.

The purpose in adding acetic acid to the stain is to enhance the staining power of the solution and to assist in prevention of loss of stain in succeeding solutions. Possible substitutes for acid fuchsin are limited to those dyes which are not precipitated by the addition of acid and are not entirely removed from the tissue by the action of phosphotungstic or phosphomolybdic acid. Masson ('29) has successfully used ponceau de xyldine.

9. *Rinse in distilled water.*

10. *Transfer to 1% phosphotungstic acid in distilled water (or 1% phosphomolybdic acid as used in Mallory's technique).* Sections are left in this reagent until the connective tissue is completely decolorized. Examination of an arteriole, readily found in most sections, is usually the criterion for perfect routine differentiation. The tunica media, consisting chiefly of smooth muscle, retains the acid fuchsin while collagenous fibers of the tunica adventitia are colorless.

The process of decolorization must be controlled according to the histological elements in the sections, because the various connective tissues differ considerably in their tendency to become decolorized; cartilage, bone matrix and dentine become destained much more slowly than collagenous fibers. Prolonged treatment with phosphotungstic or phosphomolybdic acid effects no other change than complete removal of the acid fuchsin from the connective tissue. In fact, differentiation for too short a time perhaps explains some of the discrepancies mentioned above. When conforming to a time stipulation as suggested in Mallory's original stain and some of its modifications, sometimes beautiful but incorrect results are obtained. Thus Galigher ('34) employing Mallory's technique described bone matrix as stained red by the acid fuchsin. Connective tissue (including bone) is uniformly colored by the secondary stain, anilin blue or light green, only when previously completely decolorized by the phosphotungstic or phosphomolybdic acid.

For best results fresh solution should be used for every second rack of slides, because phosphotungstic and phosphomolybdic acids rapidly lose the power of decolorizing connective tissue after they have acted upon a number of preparations. For this reason the incorporation of phosphomolybdic acid with the anilin blue, as recommended in some variants of Mallory's stain, is obviously bad.

It has been theoretically suggested that the primary stain is bound to tissue other than connective during the process of differentiation. This may or may not be true. It is a fact that in attempts to use eosin and a number of other fluorescein derivatives in place of acid fuchsin, immersion in phosphotungstic or phosphomolybdic acid results in complete removal of these stains.

11. *Rinse in distilled water.* Sections should be removed immediately so that the mordanting effect of the phosphotungstic or phosphomolybdic acid for the connective tissue is not lost.

12. *Transfer to light green (or anilin blue).*

Light green (Krall Microcolor; Eimer and Amend)	1 gm.
Distilled water	100 cc.
Glacial acetic acid	1 cc.

Since it is difficult to examine the slide in this stain, a suggested time is 5 minutes. Light green and anilin blue are mordanted to the connective tissue by the phosphotungstic or phosphomolybdic acid. With additional staining time other tissue elements may be lightly stained.

The use of light green in place of the original anilin blue was suggested by Masson ('29); on account of its clear color and low intensity of staining, it permits observation of fine protoplasmic expansions of the connective cells. Anilin blue, being more intense is better for the demonstration of reticulum fibers. The formula is:

Anilin blue (National Aniline Co.)	2 gm.
Distilled water	100 cc.
Glacial acetic acid	2 cc.

The procedure is practically the same as with light green, except that sections are left comparatively longer in the acetic acid (step 14) to obtain optimum differentiation.

13. *Rinse in distilled water*, removing the sections immediately to prevent too great loss of the connective tissue stain.

14. *Transfer to 1% acetic acid*, to remove the light green loosely bound to other than connective tissue. Sections should be left in the acid until this result is attained. Repeated inspection of a trial slide with the microscope, preferably with a substage lamp, will determine optimum differentiation. Sections may be left in the acetic acid for a considerable time without appreciable loss of the light green from the connective tissue. One to 5 minutes, depending upon the degree of overstaining which has occurred, is usually the required time.

15. *Rinse in distilled water*, removing the sections immediately.

16. *Pass through three changes of absolute ethyl alcohol followed by three changes of xylol. Mount in xylol balsam.* Probably because of the action of the acetic acid, no loss of stain occurs in the alcohols. If necessary, the time of dehydration may be prolonged to one hour. In this modification of Mallory's stain, alcohol does not act as a differentiating agent, as it does in the original; differentiation is completed in step 14.

There may be some criticism with reference to the abrupt transference of sections from an aqueous solution to absolute alcohol. Gage ('25) however states that less violent diffusion currents result by this method than in dehydration by graded alcohols; and personal observation has confirmed this statement.

Final results are identical with Mallory's specifications depending on the selection of light green or anilin blue as the connective tissue stain.

SUMMARY

In proposing the present modification of Mallory's connective tissue stain, emphasis is laid upon 1) the advantages of incorporating the orange G with the acid fuchsin rather than with the anilin blue; 2) the importance of controlling the staining and destaining processes, whenever possible, by microscopic examination rather than by time designation; 3) the necessity of varying the decolorization with phosphotungstic or phosphomolybdic acid according to the nature of the tissues being stained, since cartilage, bone matrix and dentine require a longer time than collagenous fibers.

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THE EFFECT OF LIGHT UPON SEXUAL ACTIVITY IN THE LIZARD, *ANOLIS CAROLINENSIS*, WITH ESPECIAL REFERENCE TO THE PINEAL BODY ¹

H. J. CLAUSEN AND E. G. PORIS

*Department of Experimental Biology, American Museum of Natural History,
New York, New York*

ONE PLATE (EIGHT FIGURES)

It is well known that, among numerous species of animals, sexual activity is greatly stimulated during the early spring and summer months. It is also quite evident, on the basis of a large number of experimental studies, that seasonal sexual reproduction in some animals is controlled to some degree by the relative lengths of daylight illumination and also by the intensity of illumination. The literature on sexual photoperiodicity has been recently reviewed and classified by Bissonnette ('36 a, '36 c). The receptors for this light stimulation appear to be the eyes in most animals so far studied (Bissonnette, '35, '36 b; Benoit, '34; and Ivanova, '35). However, the eyes may not be the only receptors, as was pointed out by Ivanova ('35) where she has shown that in sparrows the exposed skin alone may serve as the receptor when the eye factor is sufficiently incapacitated.

The resemblance of the pineal eye in reptiles to a visual organ has stimulated investigations regarding its sensory function. Nowikoff ('07) studied the effect of light on the pineal eye in *Lacerta agilis* and *Anguis fragilis* and showed that the movement of pigment granules in the retina was dependent upon the intensity of light. Dendy ('11) concentrated a bright

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light on the skin above the pineal eye of the rhynchocephalian, *Sphenodon punctatus*, without any resulting influence or change being noticeable. Francotte (1887) cauterized the eyes of a lizard and tested the receptive functions of the pineal eye. His results, while inconclusive, suggest the light receiving role of the pineal in the lizard *Anguis fragilis*.

In a review of the literature relative to the physiological investigations on the pineal organs, it was found that a number of workers have made no sharp differentiations between the parietal organ and the epiphysis proper. Even though there may be a question of homologous organs, it has been shown by Schmidt ('09), Tilney and Warren ('19), Boveri ('25), Krabbe ('35) and others that these structures differ considerably in their morphological development and hence should not be considered as having identical functions. Henceforth, throughout the investigations described in this paper we will use the term pineal and restrict its use herein to that specific structure of the epiphyseal complex generally referred to as the parietal eye in reptiles.

So far as the authors are aware, no investigations, as regards the specific effect of light alone upon sexual stimulation in reptiles, has been carried out. Therefore it seemed of importance to carry out a series of tests whereby this specific factor could be studied in a certain group of reptiles. Also, in view of the somewhat diverse results obtained by previous investigators as to the possible sensory function of the pineal eye in lizards, the present studies were carried out in an attempt to determine whether this organ might act as the receptor or one of the receptors for light in sexual stimulation.

MATERIALS AND METHODS

The common species of iguanid lizard, *Anolis carolinensis*, was used throughout the following series of experiments. This species of lizard was selected for experimental work because of its availability in relatively large numbers and also because this species has a prominent and well-developed pineal eye. The lizards were obtained from Thibodaux, Louisiana.

Upon arrival at the laboratory, they were placed in large stock cages in the laboratory greenhouse. After a period of 2 weeks, so as to allow the lizards to become acclimated to laboratory care, 230 adult male animals were selected from the stock cages and placed in the experimental cage. The experimental cage was divided into two compartments by means of a black glass partition. One hundred and forty of the experimental lizards were placed in the larger compartment which measured $80 \times 20 \times 30$ inches. The ninety remaining lizards were placed in the smaller compartment which measured $60 \times 20 \times 30$ inches. Seventy lizards in the large compartment and forty-five in the smaller one were pinealectomized. All animals were marked individually by severing one or more of the toes. Hence individual records could be kept throughout the course of the experiment.

The removal of the pineal eye was a comparatively simple procedure. The lizards were anesthetized with ether and placed under a binocular dissecting microscope. The pineal, which is situated on the dorsal midline surface just posterior to the eyes, was easily located by the non-pigmented central area found in the large scale over the parietal foramen. This portion of the pineal consists of a white mass of tissue lying directly under the non-pigmented portion of the scale. A sharp-pointed spearhead knife was used to separate this scale from the surrounding skin. By means of a pair of iridectomy scissors, a portion of the scale was severed and lifted off. The pineal was carried along with this overlying portion in most instances. However, in some cases it became detached and necessitated separate removal with a fine-pointed forceps.

Following the removal of the pineal eye the wound surface was cleansed with dilute alcohol and covered with collodion. The complete healing process required from 1 week to 10 days. The operated lizards were then placed in the compartments with the normal non-operated animals. The cage, consisting of two compartments, was a wood frame structure with a plate glass front and sides. The top was covered with a fine mesh screen. This cage was kept in a room with a northern exposure so that direct sunlight was entirely eliminated. The

temperature of the room was maintained at approximately 23°C. during the course of the experiment.

Beginning on November 25th, the normal and the pineal-ectomized lizard in the large compartment received additional illumination for 6 hours per day from 4.30 P.M. to 10.30 P.M. This added illumination was produced with four 80-watt bulbs made by the Schickerling Electric Products Co. An electric time switch controlled the lighting of this compartment each day. A reflector was placed over each bulb. The reflector rested on a stand so that the bottom of the bulb was 19 cm. from the screen cover of the cage. A similar lighting arrangement was set up on the control compartment except the bulb was placed within 6.5 cm. from the screen cover. In this case the light was screened off from the control compartment by covering the bulb and reflector with a piece of tin. By this arrangement, the temperature of both compartments was kept the same within $\pm 1^\circ\text{C}$. during the 6-hour illumination period. An oscillating electric fan was also placed between the two compartments so that cool air would be passed under the reflectors and hence eliminate any great increase in temperature directly below the reflector. This fan was connected to the same time switch as the lights and operated only during the 6-hour period. At 4.30 daily, the control compartment was darkened by substituting black sections of plate glass in the front and side of the compartment for the clear sections present during the ordinary daylight hours. By this method the artificial light from the adjoining compartment was kept out of the control section of the cage.

During the course of the experiment, which was continued for 50 days, the lizards were fed daily. The food consisted of flies (*Muscina stabulens*), mealworms (*Tenebrio molitor*), and larvae or moths (*Galleria mellonella*). All food was removed at 4.30 P.M. in both compartments. Small dishes of water were kept in the cage during the daylight hours and in addition the sides and floor of the cage were sprinkled with water each morning and afternoon.

When the experiment terminated, all lizards were weighed as in the beginning of the experiment. The gonads were measured and weighed at the time the animals were sacrificed. The gonads, thyroids, thymus, pituitary, as well as the pineal eye in those lizards from which it had not been previously removed, were removed before the animals were preserved in 10% formalin. The gonads were fixed in Bouin's fluid and stained with Heidenhain's iron-hematoxylin. Other tissues were also fixed in Bouin's fluid and will be considered for future study.

RESULTS

Series I. Normal lizards receiving only ordinary daylight illumination. Forty-five adult male lizards were placed in the smaller compartment on November 25th. They received only the natural daylight illumination for the following 50 days. Data on the individual weights were recorded when the experiment terminated on January 14th. At this time the testes were removed, weighed and measured and then fixed for microscopic section.

It can be seen from the data recorded in table 1 that the average weights of the lizards had remained approximately the same over the 50-day period. However, the average testis weight as well as size showed a definite increase when compared to those of the fifteen animals killed at the time the experiment began.

Microscopic sections of testes from all lizards of this series were examined and compared with sections of testes removed from lizards prior to the beginning of illumination experiment. In this series, as well as in all of the succeeding series, comparative studies of microscopic sections were based on sections taken through the largest diameter of the testes. Figure 1 shows a section through the testis of one of the lizards representative of this experimental series. As can be readily seen, the testes of this group were not in a dormant state at this time of the year. The tubules, while small as compared to the series to be described later, increased in size slightly. The intertubular spaces were narrow and very little pigment was

present. Figure 2 shows the same testis under higher magnification. In most cases no lumen was present. Spermatogonia and spermatocytes were easily recognized. In some cases intermediate stages in synizesis were present. However, no spermatids nor sperm were present.

Conclusions reached from the above series indicated that testicular activity was undergoing a progressive change during the early winter months even though a normal decrease in the ordinary daylight illumination was occurring during most of this season of the year.

TABLE 1

Data recorded to show the various weight differences in normal and pinealectomized lizards under experimental light and also under normal daylight conditions

SERIES	NUMBER OF ANIMALS	ORIGINAL WEIGHT ¹	FINAL WEIGHT	TESTIS WEIGHT	TESTIS SIZE
		<i>gm.</i>	<i>gm.</i>	<i>mg.</i>	<i>mm.</i>
Control ²	15	4.02	..	10.8	3.7 × 2.9 × 2.3
Normal (daylight)	45	3.92	3.81	12.7	3.9 × 2.8 × 2.5
Pinealectomized (daylight)	41	4.71	4.93	19.5	2.9 × 3.7 × 3.4
Normal (daylight plus 6 hours light)	70	4.44	4.48	29.0	5.3 × 3.8 × 3.3
Pinealectomized (daylight plus 6 hours light)	70	5.14	5.47	30.5	6.0 × 4.2 × 3.8

¹ Average weight of lizards at the beginning of the experiment as compared to the final weight at the 50-day termination period.

² These lizards were sacrificed and tissues fixed and examined at the beginning of the experiment.

Series II. Normal lizards, subjected to 6 hours added illumination daily. While it was noted in the preceding series that sexual activity proceeded progressively during the early winter months even though the normal daylight was very much reduced, it was thought of sufficient interest to determine whether added illumination of 6 hours per day for a 50-day period would further accelerate this activity.

The testes of seventy normal lizards subjected to 6 hours added illumination per day for 50 days were removed, weighed and measured and prepared for microscopic examination. The average testis weights, as shown in table 1, for this series are

much greater than in either the previous series or in the normal lizards at the beginning of the experiment. Figures 3 and 4 show sections through a typical testis of this series. Sixty-three lizards in this series showed approximately this increase in activity over the former series. Seven lizards showed little or no advance over those in series I. However when the weight records of these seven animals were examined, it was found that a considerable loss in weight had occurred during the 50-day period of experimentation. These seven cases showed a weight loss of from 1.0 to 1.5 gm., while the weights of the other fifty-three lizards showed no decrease, sixteen remaining the same as in the beginning of the experiment and the remaining thirty-seven showing an average increase of nearly 1 gm. per animal. This weight factor may account, to some extent, for the failure of the gonads to react progressively in the seven instances.

In the fifty-three cases a decided stimulation was noted. The tubules were nearly twice the size of those in series I, and a large lumen was present in most cases. Spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids were easily discernible in their regular order from the wall of the tubule inward toward the lumen. Sperm were quite numerous in the lumen of most tubules. Hence it can be seen from this series that increased illumination time accelerated testicular activity over and above that which was normally occurring at this time of the year.

Series III. Pinealectomized lizards receiving only normal daylight illumination. In an effort to determine whether or not the pineal eye might act as the light receptor or one of the receptors, the above two series were repeated with pinealectomized lizards. In this series forty-five adult male lizards were pinealectomized and placed in the same compartment with the lizards included in series I. In this way these animals were subjected to the same experimental conditions where only ordinary daylight illumination was used. Four of the lizards in this series died before the 50-day period terminated. Hence, only forty-one lizards were considered in the final results of this series.

Table 1 shows the average relative weights and sizes of the testes of this series. It may be noted that the increases are considerably higher than in series I where normal lizards were subjected to the same external environmental conditions but lower than in series II where normal lizards were subjected to 6 hours added light. The histological appearance of the testis in this group (figs. 5 and 6) served to show that in thirty-eight of the animals a stimulating action was present. In three cases no activity whatever was discernible. Here again, the latter three lizards showed a decline in weight for the 50-day experimental period and hence negative findings might be accounted for on this basis. However, in the thirty-eight cases the testes are at once distinguishable from the normals in series I, on the basis of increased activity. In this pinealectomized series the conspicuous feature was the marked enlargement of the tubules with a distinct thickening of the epithelium and with spermatogenesis being carried through to the formation of a relatively large number of sperm. Mitotic figures were very numerous. The lumen was also increased in size.

The results, as observed in the above group of lizards, show that the removal of the pineal eye served to bring about a more progressive state of testis activity than was found in normal lizards subjected to otherwise similar influence. However, pinealectomy alone did not increase stimulation to the same degree as the addition of 6 hours of added light stimulation.

Series IV. Pinealectomized lizards subjected to added illumination. In this series of seventy pinealectomized lizards it was of interest to determine whether or not the absence of the pineal eye would also have an additional influence on lizards when subjected to 6 hours added illumination per day for a period of 50 days.

Microscopic examinations of all testes in this group showed that a significant advance in testicular activity was noticeable when compared to the preceding series where no added illumination was used. It was noted that the sperm were

decidedly numerous (figs. 7 and 8) and that all four generations of cells were present. In eleven of the testes certain definite signs of regression were in evidence. The cytoplasm was beginning to become vacuolated, interstitial, cells were increasingly numerous and pigmentation was becoming more condensed.

The testes of the lizards in this series of experiments showed an increase in size and weight (table 1) over those of normal lizards subjected to added illumination. A percentage of testes in this series also showed signs of regression. No other evidence was present to show that in this series pinealectomy served to further activate the testis when added illumination was also given. Nevertheless the above evidence suggests the possibility of additive acceleration and it is possible that the gonads may have attained the highest degree of activity earlier than the 50-day interval used in this particular series.

DISCUSSION

The results described above show that in the lizard, *Anolis carolinensis*, testis activation may be more or less influenced by a carefully controlled environmental factor such as light. These results are consistent with other investigations on birds (Bissonnette, '31; Cole, '33; Rowan, '29 and '30; and Benoit, '34); also with certain investigations on mammalian forms (Bissonnette, '32; Allanson and Deanesley, '34; and Baker and Ranson, '32).

The species of lizard used in these experiments has a well-developed pineal eye which is also exposed to the external surface. It has been shown (Clausen and Mofshin, '36) that this organ acts as a light receptor in the *Anolis*. It has also been shown to be receptive to light of various intensities in other species of lizards (Nowikoff, '07). Nevertheless, our experiments show that the light receptive qualities of the *Anolis* pineal eye are not essential for the stimulation of the testis. Conversely, the experiments show that the obliteration of the pineal eye results in a tendency toward acceleration of activity. On the basis of these observations, it is suggested

that light reception through the pineal may act as an inhibitor of testicular activation or that an internal secretion is liberated by the pineal which in turn results, either directly or indirectly, in the production of inhibiting influences toward sexual stimulation.

It must be emphasized that while light is an important stimulus concerned with sexual activity in at least certain species of lizards, other environmental factors such as temperature and also food (Mellish, '37) may influence this manifestation as well. Furthermore, the present experiments serve to emphasize the fact that another of the important organs to be considered in studies of photoperiodicity in lizards is the pineal eye which has been shown to act as an inhibiting factor in testis activity in this species of lizard.

In previous investigations on other animals which react positively toward sexual stimulation by illumination, the eyes appear to function as the receptors for the light stimulus (Bissonnette, '35, '36 b; Benoit, '34; and Ivanova, '35). In the light of previous experiments (Clausen and Mofshin, '36) which show the eyes to be of primary importance as light receptors, experiments are being carried on in this laboratory in an effort to determine whether the eyes function as primary receptors for this stimulus toward testis activation in lizards.

SUMMARY

1. Two groups of adult male lizards were subjected to light from 80-watt bulbs for equal periods of 6 hours daily from 4.30 P.M. to 10.30 P.M. for a period of 50 days, beginning November 25th. One of the above groups consisted of seventy normal lizards while the other group of seventy lizards was pinealectomized.

2. Two control groups of forty-five lizards each, received only normal daylight illumination up to 4.30 P.M. daily, after which time they were placed in darkness. Here again one group was made up of normal lizards while the other group was pinealectomized.

3. A microscopic study was made of the testes of all the lizards at the termination of the experiment. The results of these experiments show that added illumination serves to induce an increase in spermatogenic activity for the period involved. The facts also indicate that the removal of the pineal eye results in an increased testicular stimulation. Hence this organ is not one of the primary avenues of light reception serving toward testis activation.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

1 Photomicrograph of a section of the testis showing the seminiferous tubules of a normal lizard subjected to ordinary daylight for 50 days, beginning November 25th. $\times 75$.

2 Photomicrograph of a section of the above testis showing solid nature of the tubule and absence of late spermatogenic stages. $\times 335$.

3 Photomicrograph of portion of the testis of a normal lizard treated with 6 hours of added light per day for a period of 50 days. $\times 75$.

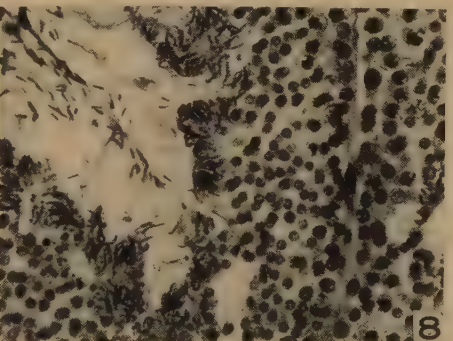
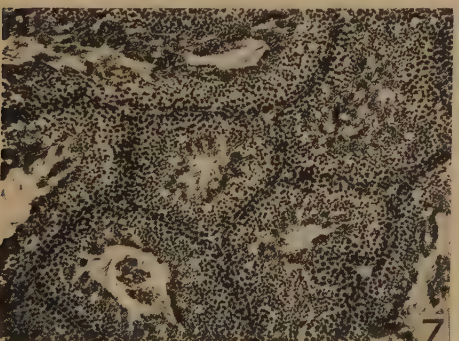
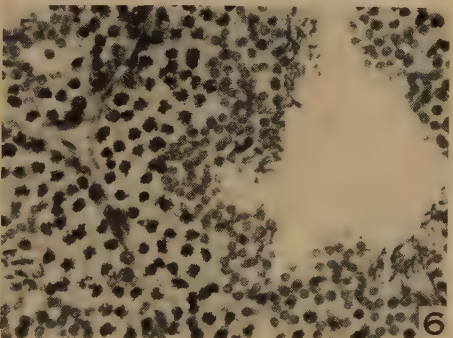
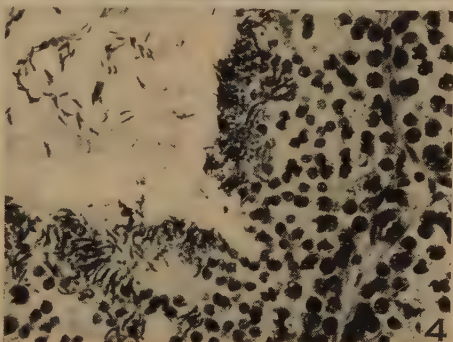
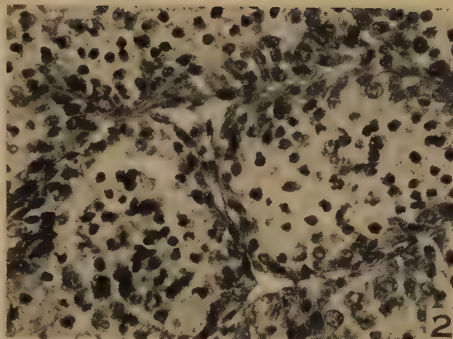
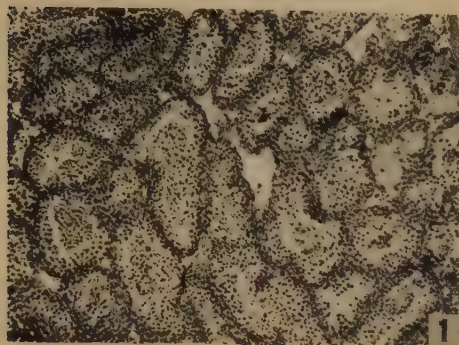
4 Photomicrograph of the above testis (fig. 3) showing active mitosis and the late stages of spermatogenesis up to and including a number of mature sperm in the lumen. $\times 335$.

5 Photomicrograph of a section of the testis of a pinealectomized lizard subjected to only ordinary daylight illumination for a 50-day period. $\times 75$.

6 Photomicrograph of a portion of one of the tubules shown in figure 5, showing active mitosis and a few sperm. $\times 335$.

7 Photomicrograph of portion of testis of a pinealectomized lizard treated with 6 hours added illumination for a 50-day period. $\times 75$.

8 Photomicrograph of portion of seminiferous tubule of testis shown in figure 7. Note the large number of sperm in lumen, active mitosis, and all late stages of spermatogenesis. $\times 335$.



THE SCHWEIGGER-SEIDEL SHEATH (ELLIPSOID) OF THE SPLEEN

OTHMAR SOLNITZKY

*Department of Anatomy, Georgetown University School of Medicine,
Washington, D. C.*

THREE PLATES (SIX FIGURES)

Of the many problems of the finer structure and function of the vascular system of the spleen, one of the most intriguing has been that of the histological nature and function of the Schweigger-Seidel sheath. In spite of the many papers dealing with this subject which have been written since this structure was first described nearly 80 years ago, there is still no agreement as to its essential histological makeup and function. Most modern textbooks in histology give only a vague description of the Schweigger-Seidel sheath. Addison ('32) makes no mention of it in his edition of Piersol's Normal Histology. Cowdry ('34) states that the wall of each penicillar artery "becomes locally thickened in a manner not found elsewhere in the vascular system." Bremer ('36), Jordan ('31), and Smith ('34) refer to the sheath briefly as a concentric or ellipsoidal concentration of reticular connective tissue. Cajal ('33) speaks of it as a reinforcement of the adventitia of the enclosed vessel with longitudinal fibers. Schaffer ('33) regards the sheath as a muscular structure and compares it to an arterio-venous anastomosis as found in the cutaneous glomus. Maximow and Bloom ('34) describe the sheath as "a compact mass of concentrically arranged elongated nuclei (probably reticular cells) and longitudinal fibers which continue with the reticular fibers of the red pulp." Petersen ('31) describes the sheath as a syncytial structure. Sharpey-Schäfer ('34) refers to the sheath as a "spindle-shaped investment of concentric lamellae of connective tissue with numerous lymphocytes in the meshes of the tissue."

There is also no agreement as to the nature of the blood vessel passing through the sheath. By some the blood vessel is called the 'sheathed artery' and by others it is regarded as a capillary.

It is the aim of this paper to prove that the Schweigger-Seidel sheath is a part of the reticulo-histiocytic system and that the contained axial vessel is a capillary and not an artery.

HISTORICAL

Billroth (1857) was the first to observe the sheath in the spleen of birds. Key (1861) first saw it in the spleen of mammals. Schweigger-Seidel (1863) gave the first extensive description of this structure in the pig, dog, cat, and man, and called it 'Kapillärhülse.' He also described communications between the meshes of the sheath and its axial vessel and hence considered the entire structure as a sort of filtration apparatus. Müller (1865) gave the first comprehensive description of the sheath in fishes, reptiles, birds, and carnivores. He was also the first to apply the term 'ellipsoid' to this structure. Müller interpreted the sheath as a fibrous thickening of the adventitia of its axial vessel and as the termination of the splenic nerves. According to Kyber (1870) the sheath is a loosened adventitia infiltrated with lymphocytes; i.e., identical in structure with the malpighian corpuscle. He also denied any communication between the capillary lumen and the meshes of the sheath. Bannwarth (1891, 1893) failed to find the sheath in the human spleen, but described it in the cat's spleen, especially in young animals. He considered the sheath as a growth center for the splenic pulp and hence important only during the embryonic period. Sokoloff (1888) who studied only the human spleen denied the existence of the sheath. Hoyer (1892) made no statement as to the histological structure of the sheath, but considered it as a mechanical provision against tearing of the axial vessel from increased intra-arterial pressure or compression from without. He explained the appearance of blood cells within the sheath as due to postmortem rupture of the axial vessel. Kultschitzky (1882, 1895) described the sheath as a compact mass

of leucocytes. Carlier (1895) considered the sheath as a compact mass of reticular fibers containing connective tissue cells. Whiting (1897), von Ebner (1899) and Janossik ('03) found smooth muscle cells, which they considered as essential constituents of the sheath. Mall ('00) first proposed the theory that the sheath acts as a one-way valve, preventing the reflux of blood from the venous into the arterial side.

The later literature will be dealt with in the discussion.

MATERIAL AND METHODS

The material used in the present study consisted of 4 spleens of the rabbit, 6 of the dog, 6 of the cat, 3 of the sheep, 4 of the pig, 2 of the calf, and 7 of man. Of human spleens, three were of newborns and four of adults. The spleens were divided into three groups.

One group of spleens was examined histologically in the contracted state after removal from the body. The second group of spleens was examined after perfusion through the aorta or the splenic artery with oxygenated Ringer solution warmed to 38°C. In the case of the rabbit, dog and cat, the perfusion was done with the spleen in situ, while the animal was still under anesthetic. The perfusion lasted from 3 to 6 hours, depending upon the size of the spleen. The perfusion was followed by the fixing fluid. The following fixing fluids were used: 10% formol in normal saline, Susa, Regaud, Maximow, Bouin, and Carnoy. Several blocks of tissue were removed from each spleen, dehydrated, and embedded by the methyl-benzonate-celloidin method. Serial sections were made from each block. The sections were divided into groups and stained by the following methods: Harriss's hematoxylin and eosin, Weigert's iron hematoxylin with picro-fuchsin or picro-indigo-carmin, Heidenhain's iron hematoxylin counterstained with aurantia or benzolicht bordeaux, Mallory's triple stain (Andersen's modification), Heidenhain's azan method, Masson's triple stain, Weigert's elastic stain, orcein, and the Bielschowsky-Maresch silver method. The third group consisted of spleens of dogs and cats which were injected intravenously with trypan blue and India ink. The trypan blue

used was a 0.5% solution in boiled distilled water. The amount of this solution injected was calculated on a basis of 3 to 4 cc. per 1000 gm. of body weight. The India ink solution consisted of a 5% suspension in sterile normal saline. From 20 to 40 cc. were injected intravenously. After definite intervals, as described below, these spleens were perfused with Ringer solution, followed by Carnoy or Susa, and then placed in the same fixing fluid. In all cases serial sections were made, as single sections were found to be of no value at all.

PERSONAL OBSERVATIONS

The following personal observations are based almost exclusively on the study of perfused spleens, since unperfused, contracted spleens were found useless for the investigation of the finer structure of the sheath due to the close packing of its constituent cells and the density of the surrounding splenic pulp.

Upon following the follicular artery as it emerges from the splenic corpuscle, it was found to divide, as a rule, into two to six branches, called penicillar arteries or arteries of the red pulp. These, contrary to the usual textbook descriptions did not run parallel to each other, but pursued a radiating course from their point of origin. Only an examination of serial sections, especially those stained with iron hematoxylin, could bring out this point. Each penicillar artery (fig. 4) has a definite, delicate intima, consisting of endothelium and a thin subendothelial layer of delicate collagenous fibers, with no elastica interna; a thin media of two or three layers of smooth muscle cells with no elastica externa; and a still thinner adventitia of collagenous and elastic fibers. The lumen of the penicillar arteries is very conspicuous as compared with that of the axial vessel of the sheath (figs. 3, 4). Each penicillar artery, after following a more or less straight course, finally branches, as a rule, into two to three branches which immediately lose the structural characteristics of an artery to become a capillary (fig. 2). The majority of these capillaries

become surrounded by a Schweigger-Seidel sheath immediately upon their appearance. There are, however, many capillaries arising as branches of a penicillar artery which are not associated with a sheath. In the rabbit spleen, in spite of a diligent search of many serial sections, sheaths were never found. This finding agrees with those of Mills ('26) and von Herrath ('35). With the exception of the rabbit, the sheath was found in all the spleens examined.

Topographically, the sheaths were found throughout the splenic pulp, but never in the splenic corpuscles (fig. 1). As a rule, they had an intimate anatomical relation to the venous sinuses. They may be in close proximity to a venous sinus, project into it, or be surrounded by several venous sinuses (fig. 3). However, in no case was there ever found a direct communication between a sheath and the adjacent venous sinuses, a small lamina of splenic pulp always separating the two.

Most often only one sheath was seen at the termination of a penicillar artery. Occasionally, from two to as many as five vessels were found to be associated with a single sheath. Sometimes, two to three sheaths were seen arranged serially upon a single branch of a penicillar artery. The axial vessel of the sheath was occasionally found divided into two or more branches, each branch being surrounded by a prolongation of the sheath. In this way more or less complex sheaths were formed.

The size of the sheath varied considerably in the various species examined. It was largest in the spleen of the pig, dog and cat. It was smallest in the spleen of the calf and man. Many differences in size were also found in the sheaths of the same specimen. There was no relation between size of the sheath and the age of the individual from which the specimen was obtained, as determined by examination of the human spleens of newborns and adults. As a rule, the sheath was smaller and more compact in the contracted, unperfused than in the dilated, perfused spleen.

The shape of the sheath was even more variable than its size, not only in different species, but also in the same specimen. In general, two varieties of shape were observed: the elongated or almost tubular, seen in the spleen of the dog and man; and the spherical or ellipsoidal shape, seen in the spleen of the pig and the cat.

The structural components of the sheath were found to be two in number: fibers and cells.

The fibers, making up the framework of the Schweigger-Seidel sheath, were invariably found to be reticular fibers. These fibers stained black with the silver method and blue with the azan method. With picrofuchsin they barely stained a very light pink color in contrast with the deep red stain seen in the collagenous fibers of the trabeculae, capsule, and the larger blood vessels. Picro-indigocarmin did not stain them at all. The fibers formed a network enclosing meshes which were small in the contracted, but larger in the perfused spleen. The fibers are best seen in perfused spleens of animals that had been previously injected with trypan blue or India ink and killed 24 hours afterward, when the sheath was seen almost freed of its cellular elements as the result of the migration of the latter into the surrounding splenic pulp. The fibers were arranged neither concentrically nor longitudinally, as claimed by various investigators, but form a closely-knit network which persists as such after long-continued and even forcible perfusion. Internally, the fibers are continuous with a circularly disposed layer of reticular fibers immediately surrounding the contained axial vessel. At the periphery of the sheath, the reticular fibers are directly continuous with the reticular fibers of the splenic pulp.

Examination of many serial sections of perfused spleens, stained with orcein or Weigert's elastic stain, has never shown the slightest trace of elastic fibers within the confines of the sheath. Invariably, the elastic fibers in the wall of the penicillar artery ceased abruptly at the beginning of the sheath. Smooth muscle cells also were never found, even though a diligent search was made for them in sections of spleens in

which the sheath had been rendered free of its contained cells by injections of trypan blue or India ink. Such sections, stained with picrofuchsin and picroindigocarmin, also showed no trace of collagenous fibers.

The cells found in the Schweigger-Seidel sheath were of two kinds: those which were found constantly in the sheaths of all specimens examined as characteristic constituents of their structural makeup, and those which were found only occasionally. Among the latter were red cells, whole or fragmented; lymphocytes; and polymorphonuclear leucocytes. The latter cells did not form a characteristic and constant finding, but merely represented cells of passage and need not be discussed further.

The appearance of the characteristic cells of the sheath depended entirely upon the technique of preparing the sections and upon the degree of distention of the spleen. It was practically impossible to study these cells well in contracted spleens as they were too closely packed in such specimens to distinguish their cytological characteristics. In preparations obtained from perfused spleens fixed in 10% formol, the outlines of the cells were ill-defined and the cells seemed to form a syncytium. This finding explains the usual description of the cytoplasmic constituent of the sheath as a syncytium. In order to determine definitely the presence or absence of a syncytial cytoplasmic network, formol as a fixative was discarded early in the present investigation and replaced by the fixing fluids of Carnoy, Regaud, Susa, Bouin and Maximow. In all preparations of spleens fixed by one of the five above-mentioned fixing fluids, it was not at all difficult to observe that the characteristic cells of the sheath were discrete and did not form a syncytium. Toward the center of the sheath, the cells were founded or ovoid, but toward the periphery of the sheath there was observed a gradual transition into the stellate reticular cells of the splenic pulp. Around the axial vessel, the sheath cells were, as a rule, closely packed, whereas toward the periphery the arrangement was looser. However, no definite pattern of cellular arrangement was seen. In many

cases, the cells were either distributed irregularly or clumped together at some point. This was especially true of the sheath cells in the preparations from the human spleens.

The cytoplasm of the sheath cells stained well with acid dyes and showed a slight, fine granulation. The nucleus was round or oval, depending upon the shape of the cell, and more or less vesicular, the chromatin being disposed in the form of small granules. In no preparation could any distinction be made between the sheath cells and the reticular cells of the pulp.

The cellular picture, however, changed dramatically in preparations obtained from spleens which had been injected with trypan blue or India ink.

Within 7 to 10 hours after such injections, the sheath cells were found invariably filled with granules of trypan blue or ink. At the same time the cells appeared much larger, spherical and, in many cases, small pseudopodial processes were seen projecting from the free surface of the cells. In preparations made 24 hours after the injection, most of the sheath cells had migrated out of the meshes of the sheath into the surrounding pulp, where they could be seen scattered singly or in small groups, close to a venous sinus. As the result of this massive migration of the sheath cells, the sheath itself could be seen as a network of reticular fibers with almost empty meshes. At no time was the sheath meshwork seen without at least a few cells. In preparations made from 5 to 8 days after the injection of trypan blue or ink, the sheaths were found almost wholly devoid of granule-laden cells. Instead, the sheath was seen to be the seat of a marked hyperplasia, the newly formed cells presenting the identical histological characteristics that were exhibited by the sheath cells of spleen into which ink or trypan blue had not been previously injected. In the hyperplastic mass, several mitoses were seen. There was no evidence that the newly formed cells were derived from the endothelium of the axial vessel, or that they had migrated into the sheath from the surrounding pulp. All evidence pointed to the interpretation

that the newly formed cells were derived by mitosis from the cells which had not migrated out of the sheath into the splenic pulp.

Cytoplasmic stains and silver impregnation showed also that the axial vessel is not an artery but a capillary, consisting of an endothelial tube surrounded by a thin layer of circularly disposed reticular fibers which, peripherally, are continuous with the reticular fibers of the sheath. The endothelial cells were elongated, their long diameter being disposed parallel to the course of the vessel. The nuclei were relatively large and projected conspicuously into the lumen (fig. 3). At the beginning of the sheath, the circular reticular fibers surrounding the axial vessel were usually arranged in two to three rows, but within the confines of the sheath only one row of reticular fibers was seen. Moreover, the reticular fibers immediately surrounding the axial vessel were more delicate than those constituting the framework of the sheath itself. There was no evidence of any permanent stomata in the endothelial lining of the axial vessel communicating directly with the meshes of the sheath network. On several occasions, red cells and granulocytes were seen passing through the endothelial wall, but at the point of exit from the vessel the migrating cell was constricted, showing that the process of cellular migration through endothelium here is not different from that seen in capillaries elsewhere in the body.

DISCUSSION

The various views that have been advanced to explain the structure of the Schweigger-Seidel sheath may be grouped as follows:

1. The sheath is a nervous structure receiving the terminal arborizations of the splenic nerves (Müller, 1865). This view is untenable as there is neither histological nor physiological evidence to support it.

2. The sheath is a contractile muscular organ (Bannwarth, 1891; Whiting, 1897; von Ebner, 1899; Janossik, '03; Schaffer, '33). Since in the present study no smooth muscle cells were

ever found in the large number of serial sections of different spleens, stained with appropriate methods, this view must also be abandoned. The same conclusion was reached by Mills ('26), Riedel ('32) and others.

3. The sheath is a localized thickening of the adventitia of the axial vessel (Schweigger-Seidel, 1862; Kyber, 1870; Hoyer, 1892; Carlier, 1895; Weidenreich, '01; Sobotta, '14). This interpretation must also be discarded as the axial vessel of the sheath has been shown to be a capillary distinct from the enclosing sheath. The independent character of the axial capillary is well shown in the spleen of the rabbit, where in spite of the absence of a sheath, it presents exactly the same structural characteristics as the axial capillary of the spleen of animals that are provided with sheaths.

4. The sheath is a localized infiltration of the perivascular connective tissue with lymphocytes (Kyber, 1870; Hartmann, '30), granular leucocytes (Kultschitzky, 1862, 1895), sessile phagocytic cells (Tait, '27), sessile white cells (Neubert, '22), monocytes or endothelial phagocytes (Mills, '26) and endothelial cells (MacNeal, '27). Although lymphocytes and granulocytes are occasionally found in the Schweigger-Seidel sheath, they do not form its essential cellular constituent. In addition, they do not characteristically take up particulate matter from the circulating blood. The terms 'sessile phagocytic cells,' 'sessile white cells' and 'endothelial cells' (as used by MacNeal) are too vague as to terminology. The term 'endothelial phagocytes' used by Mills is partly correct in that the cells of the sheath are intensely phagocytic. However, Mills uses the terms monocytes and endothelial phagocytes interchangeably. In hematological nomenclature these two terms do not signify the same thing. The use of the term 'endothelial' in this connection is objectionable since endothelium is not characterized by intense phagocytosis of particulate matter. In discussing the origin of the phagocytic cells of the sheath, Mills states that they are "presumably a modified type of endothelium" derived from "the cells at the termination of the arteriole." However, he does not make clear just what the nature of these cells is.

As none of the views discussed above takes into account the actual structural characteristics of the sheath, there remains only one interpretation that is in accordance with the factual findings of the present study; namely, that the sheath is a localized condensation of splenic pulp tissue, consisting, as it does, of the same structural elements: reticular fibers and intensely phagocytic reticular cells. This view is supported by the findings of Hueck ('28), Jaeger ('29), and Li, Garven and Mole ('29).

The axial vessel of the sheath has been considered as an artery or precapillary artery by almost all investigators and by practically all authors of textbooks of histology. Only Schweigger-Seidel (1862, 1863) and Petersen ('31) have definitely and consistently termed the axial vessel a capillary. The findings of the present investigation leave no doubt that the axial vessel of the sheath is a capillary, consisting of an endothelial tube surrounded by a single layer of circular reticular fibers with no elastic or muscular fibers. Several investigators (Schweigger-Seidel, 1863; Hoyer, 1892; Phisalix, 1885; Greschik, '15; Staemmler, '25; Mills, '26; Oberniedermayr, '26; Li, Garven and Mole, '29; Hartmann, '30; Teitel-Bernard, '31) have claimed that the axial vessel of the sheath is perforated. Mills, for instance, states that such perforations can be determined by the appearance of the ink perfused sheaths and by the observation that at certain points along the vessel wall, where it seems to lose its continuity, the nucleus of an endothelial cell is averted. Teitel-Bernard supports his contention that the endothelium has preformed stomata by the observation of the passage of red cells through the endothelial wall, but his figure 3 shows that such passage of red cells is simply a case of diapedesis. Although evidence was sought in the present investigation for any permanent preformed communications between the lumen of the sheathed capillary and the meshes of the sheath, none was found. In both the contracted and the dilated spleens the lumen of the capillary was found to be remarkably constant. An interesting observation which supports the view represented here,

namely, that the meshes of the sheath are not in direct communication with the vascular system through preformed stomata is that of Passalacqua ('32). This investigator injected the arterial system with a 2% aqueous solution of trypan blue, then made sections 100 μ thick, dehydrated them completely in absolute alcohol, and finally immersed them in oil of wintergreen. All the tissues became transparent and only the vessels filled with the dye were visible under the microscope. Close examination of these preparations revealed no trace of ellipsoids. The spleens employed by Passalacqua were of human adults.

As regards the function of the Schweigger-Seidel sheath, the following theories have been advanced:

1. The sheath is a one-way valve (Stieda, 1862; Mall, '00). The observation of Stieda that the spleen cannot be perfused from the venous side has been corroborated by many investigators. Mall was the first to explain this finding as due to a one-way valve action of the sheath. This view was further elaborated by Tait and Cashin ('25) and since then most investigators have considered the sheath as a valve preventing backflow from the venous into the arterial side. Mills ('26) found that Stieda's finding was true of only the contracted spleen. By perfusing a dilated spleen, Mills succeeded in obtaining a reverse flow through the ellipsoids. However, there is very little evidence in favor of the assumption that the sheath is a one-way valve. In the rabbit, where there are no sheaths, there is as much and even greater difficulty in obtaining a reverse flow as in spleens that are provided with sheaths. Moreover, in the spleens of dogs, cats, pigs, etc., not all the capillary branches of a penicillar artery have sheaths and yet reverse flow is as difficult to secure through them as through sheathed capillaries. As the splenic corpuscles are wholly resistant to reverse flow, even when such flow is obtained through the sheathed capillaries, a valvular action may more logically be attributed to them than to the sheaths. Finally, the lumen of the sheathed capillary is exceedingly constant in the contracted and perfused spleen.

This would hardly be the case if the sheath had a very important valvular action. It is more logical to suppose that the resistance to reverse flow is due to some other factor, such as the compression of the capillaries and arterioles by the venous sinuses, distended by the perfusing fluid (Hueck, '28). As the capillaries of the spleen tend to collapse unless kept distended by an arterial pressure greater than the pressure of the fluid medium around them, it is hardly necessary to attribute to the Schweigger-Seidel sheath a special one-way valve action to explain the resistance to reverse perfusion.

2. The sheath is a capillary regulator (Heidenhain, '28; Oberniedermayr, '26; Henschen and Reissinger, '28). Heidenhain supposed that when the spleen contracts, the sheaths also contract as the result of their natural tonus and so protect the splenic pulp from a sudden overflowing when the blood pressure rises abruptly. Henschen and Reissinger, like Schaffer ('33) considers the sheath as a vasomotor organ, an autonomic neurovascular structure, which regulates local blood flow and blood pressure. They compare them in function to the cutaneous glomus described by Masson. Although the sheath may somehow regulate the lumen of the capillary, there is neither histological nor physiological evidence to indicate the exact nature of this mechanism.

3. The sheath is a growth center for the splenic pulp (Bannwarth, 1891, Greschik, '15; Staemmler, '25). According to this view the sheath by constant proliferation contributes to and maintains the substance of the splenic pulp. The supporters of this view also maintain that the sheath is important functionally only during the embryonic period. This view is hardly tenable as the examination of spleens from human newborns and adults has shown there is no significant difference in appearance, size, or distribution.

4. The sheath is a part of the reticulo-histiocytic system. This is the only interpretation of the function of the Schweigger-Seidel sheath that harmonizes with the facts. Its constituent cells, like those of the reticulo-histiocytic system throughout the body, are characterized by a selective and

intense phagocytosis of particulate matter, by their rapid mobilization into migratory, ameboid phagocytes under the influence of injected vital dyes or India ink, and by their remarkable capacity for proliferation and regeneration.

SUMMARY AND CONCLUSIONS

1. The Schweigger-Seidel sheath is a localized condensation of splenic pulp, consisting of a network of reticular fibers and intensely phagocytic reticular cells.

2. The sheath does not contain smooth muscle cells, collagenous or elastic fibers.

3. The sheath is not an embryonic structure, but functions throughout life.

4. The sheath has no active regulatory function.

5. The sheath forms an integral part of the reticulo-histiocytic system.

6. Regeneration of the sheath occurs through mitotic division of its own component reticular cells.

7. The axial vessel of the sheath is not an artery, but a capillary and should be designated the 'sheathed capillary.'

8. The sheathed capillary has no preformed stomata and is hence not in direct communication with the meshes of the sheath.

9. No ellipsoids occur in the spleen of the rabbit.

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PLATE 1

EXPLANATION OF FIGURES

1 Photomicrograph of a section of the dog's spleen perfused for 3 hours with oxygenated Ringer solution warmed to 38°C. Stained with Heidenhain's iron hematoxylin. On the right is seen the follicular artery, FA, entering the splenic follicle, F. In the surrounding pulp are seen several Schweigger-Seidel sheaths, S. $\times 80$.

2 Photomicrograph of a section of the calf's spleen perfused for 4 hours with oxygenated Ringer solution warmed to 38°C. A penicillar artery, PA, is seen on the right dividing into two branches which, after a short course, become capillaries and enter a Schweigger-Seidel sheath, S. Notice the close proximity of the upper sheath to the venous sinus, VS, from which it is separated by only a thin lamina of pulp tissue. The upper sheath is cut longitudinally; the lower, obliquely. $\times 300$.

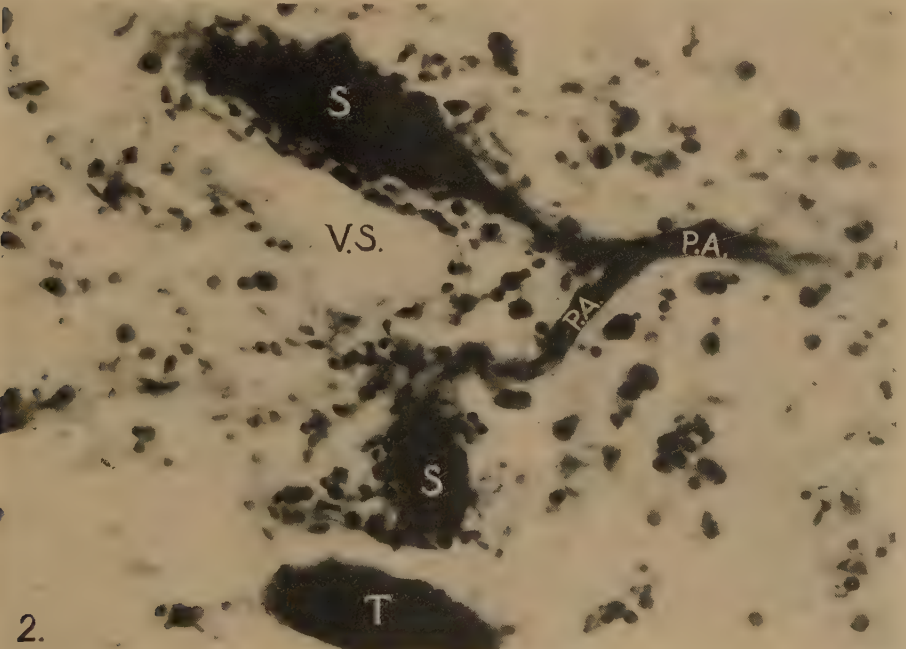
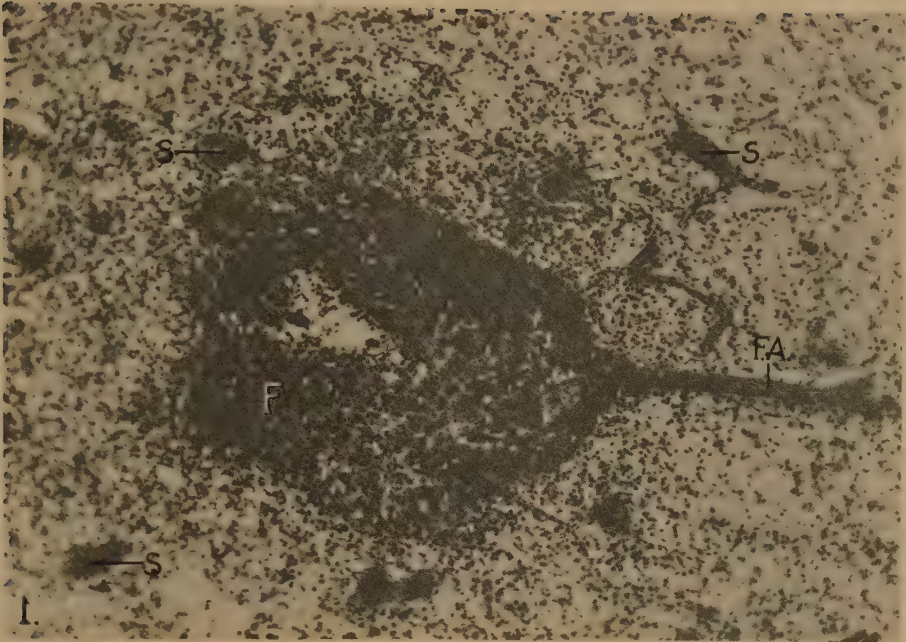


PLATE 2

EXPLANATION OF FIGURES

3 Photomicrograph of a section of human spleen perfused for 6 hours. Sheath, S, is cut transversely and almost completely surrounded by venous sinuses, VS. In the meshes of the reticular network of the sheath are seen the reticular cells scattered irregularly. In the center of the sheath is the capillary, SC, its lumen almost obliterated by two projecting endothelial cell nuclei. $\times 400$.

4 Photomicrograph of a section of the cat's spleen perfused with oxygenated Ringer solution warmed to 38°C. Azan stain. Two penicillar arteries, PA, are shown cut transversely. Notice definite arterial wall and conspicuous lumen. To the right is seen a terminal capillary, C, emerging from a sheath and emptying into a venous sinus, VC. T, trabecula. $\times 400$.

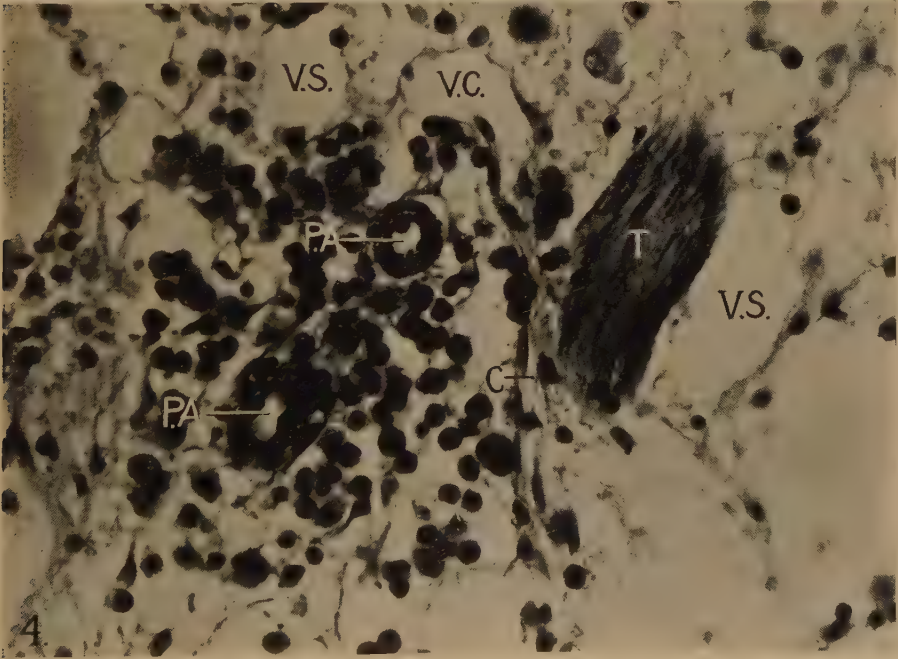
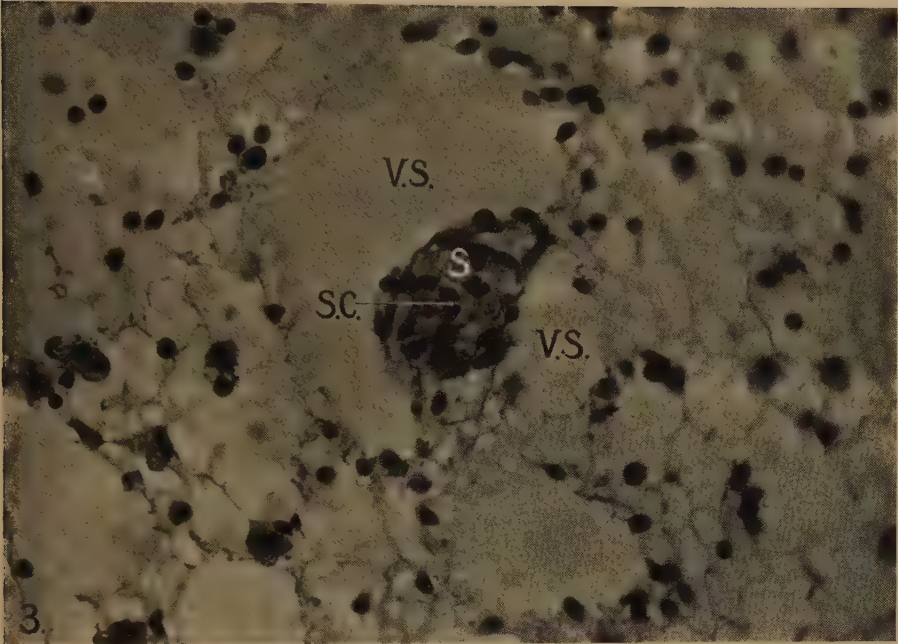
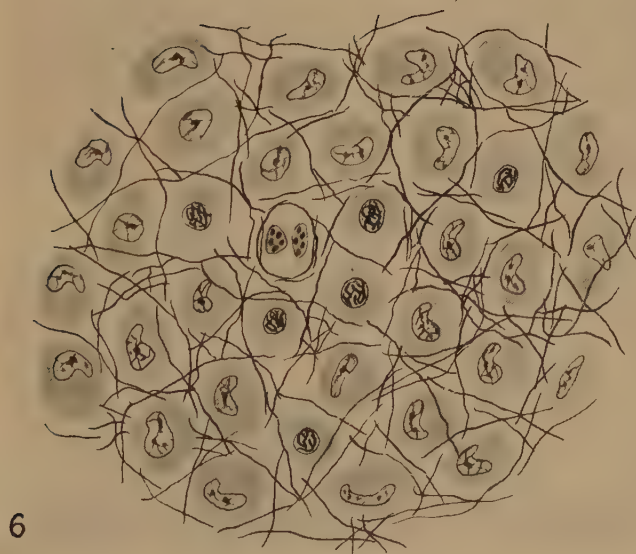
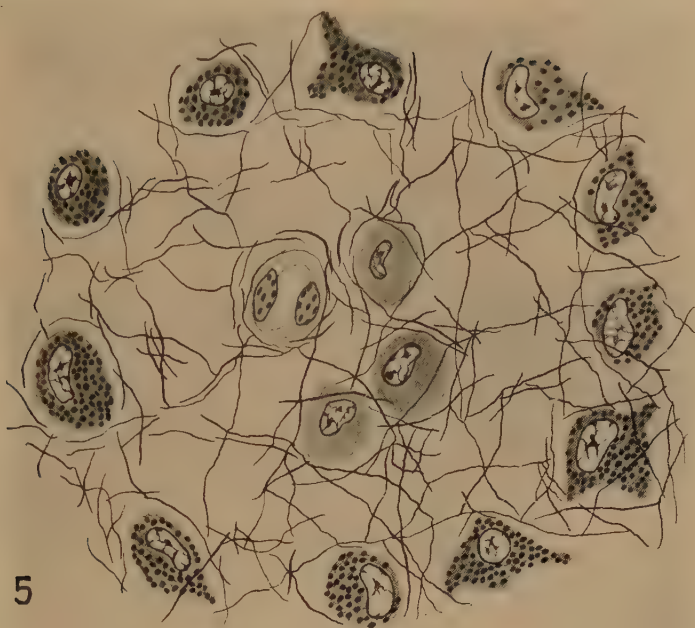


PLATE 3

EXPLANATION OF FIGURES

5 A drawing of a section of the dog's spleen, removed 22 hours after intravenous injection of trypan blue. Notice that most of the cells, filled with granules of the dye, are migrating out of the sheath, so that the reticular framework of the latter can be seen clearly. In the center is the sheathed capillary. Bielschowsky-Maresch impregnation. $\times 1000$.

6 A drawing of a section of the dog's spleen, removed 7 days after intravenous injection of trypan blue. The sheath is freed of granule-laden phagocytes. There is a marked hyperplasia of the reticular cells. Bielschowsky-Maresch impregnation. $\times 1000$.



RENAL AGENESIS IN A RABBIT

WARREN C. CORWIN

Fellow in Experimental Surgery, The Mayo Foundation, Rochester, Minnesota

Congenital absence of one kidney is by no means a rarity in man, and there is an ever increasing number of case reports and reviews of the subject. The incidence is given variously as follows: Menzies, 1:900; Motzfeldt, 1:450; Naumann, 1:700; Hepler, 1:1600; and Collins (581 cases), less than 1:1000. Despite the fact that within recent years there have appeared several reports of solitary kidney in the rat, observations on malformations of the urinary tract in small laboratory animals are still given only very sparingly. Jaffé believed that this may be attributed to the fact that this type of malformation is frequently incompatible with life and that, therefore, such animals are not examined.

Because of the apparent rarity of anephrogenesis in the rabbit, the present case seemed worthy of brief description. No previous examples of this anomaly have been met with in routine necropsy material in this laboratory during the past 20 years. A review of the literature yielded but one previous report, that of Harrison. Jaffé mentioned a case reported by Strecker, but he did not give the reference and I have been unable to find it. Harrison's case was one of pure agenesis, whereas Strecker's was combined with absence of the right half of the uterus and vagina.

In the present case the rabbit, a female, weighed approximately 3 kg. Artificial exstrophy of the bladder had been carried out for studies on ureteral catheterization. At the time of operation and following it only one ureteral orifice could be identified. At necropsy no vestige of the right kidney could be found. There were no remnants of the right ureter or right

renal vessels. The right adrenal gland was present and appeared to be normal. There was no evidence of arrested development of the generative organs. The animal was pregnant (estimated at 3 weeks) and each horn contained four fetuses. The left kidney was quite normal though considerably enlarged, weighing 14 gm. as against the normal of 8 gm. for a rabbit of corresponding size.

As usually stated there are three possible errors of development which may result in agenesis of the kidney: 1) The ureteric bud may fail to arise, 2) the bud may appear but fail to reach the metanephrogenic tissue, or 3) the metanephrogenic cap may fail to develop. Boyden, however, from study of a 10-mm. human embryo with unilateral renal agenesis and from experimental studies on obstruction of the wolffian ducts in young chick embryos was of the opinion that no evidence has as yet been presented which justifies the theory that absence of the kidney is due to lack of appearance of the metanephric blastema. He concluded that it is due to retardation in development of the ureter, to embryonic agenesis of the ureter, or to arrest in development of the parent tissue of the ureter.

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EXPERIMENTAL STUDIES OF THE THYROID

I. EFFECTS OF THYROIDECTOMY ON REPRODUCTIVE ORGANS IN MALES OF AN ANNUAL-BREEDING GROUND SQUIRREL¹

MOSES ZALESKY

Hull Zoölogical Laboratory, The University of Chicago

AND

L. J. WELLS

Department of Anatomy, University of Missouri

ONE PLATE (TWO FIGURES)

INTRODUCTION

With the gradual accumulation of knowledge regarding endocrine interrelationships, it is becoming increasingly apparent that the thyroid gland may constitute a factor of some significance in sexual development and activity. The precise role of the thyroid gland as related to reproductive processes, however, is still largely within the realm of speculation. It is not altogether improbable that the thyroid-gonad relationship may eventually be shown not to be identical in the various species subjected to experimental investigation. The investigator must bear in mind that, in working with domesticated laboratory animals, he may be dealing with a variety of confusing, perhaps specific, deviations from the fundamental pattern. Hence, from the viewpoint of basic biological research, a study of the thyroid-sex relationship in a wild, annual-breeding mammal is especially desirable.

The male ground squirrel *Citellus tridecemlineatus* used in our studies has an annual sexual cycle in which a brief breeding period (April to May) alternates with an extended phase

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of low sexual activity (Moore et al., '34). From June to January the male reproductive system exhibits virtual quiescence characterized by small aspermatic testes which are withdrawn from the scrotum into the abdominal cavity. Secretion of testis hormone is reduced to an insignificant quantity and, consequently, the accessory reproductive organs are involuted. Renewed sexual activity becomes detectable by December or January in laboratory males, while it appears about a month later in field animals. Continued development results in breeding capacity by March in laboratory animals, and by April in field males. This alternation of high sexual activity with sexual quiescence in males of this species is thought to be dependent, at least in part, upon seasonal variation in the quantity of gonadotropic hormone elaborated by the anterior pituitary gland (Wells, '35).

A study of gross and microscopical seasonal changes in the thyroid gland of this species (Zalesky, '35) revealed that whereas hypertrophy and hyperplasia take place in the thyroid gland of either sex during the breeding period and are particularly prominent in the female during pregnancy, the thyroid-sex interrelationship is not entirely clear. It appears to be superimposed upon, and therefore obscured by, an essential thyroid cycle probably associated with the special adaptation of hibernation and consequent seasonal changes in metabolism. Further experimental analysis was therefore indicated.

The series beginning with this paper represents an attempt to discover the part which the thyroid gland plays in the reproductive cycle of *Citellus tridecemlineatus*. The first line of attack decided upon was to thyroidectomize ground squirrels during the period of sexual quiescence, and to determine whether this treatment would affect the reproductive cycle. The male was chosen first since its annual sequence of sexual activity remains basically expressible during captivity.

Acknowledgments are gratefully extended to Dr. Carl R. Moore, for his continued interest in the project and critical

reading of the present manuscript; to Mr. Lawrence Tower for acting as anaesthetist; and to Mr. Morris Goroff for his invaluable assistance in procuring the animals from the field.

TECHNIQUE

In 1934 and 1935, thyroidectomy was performed on 120 male ground squirrels. An additional fifty untreated males were kept as unoperated controls. Usually three or four operated animals were housed together with one or two unoperated males, hence both experimental and control animals lived under practically identical environmental conditions. The animals were usually operated during October and November, i.e., during the phase of low sexual activity, and sacrificed in March or April, i.e., during the period in which the reproductive organs of males under laboratory conditions are normally well developed and physiologically ready for breeding. Only animals dying of cachexia or of other postoperative complications were killed as early as February or the last week in January. Animals dying before January 31 were disqualified for the purpose of the present investigation since even in the normal controls the testes and reproductive accessories were low at that time of the year. After extensive experimentation, the following operative technique was found most satisfactory.

The animal is subjected to deep ether anesthesia, placed on its back and tied to the operating board by its legs. An additional tie looping the upper incisors and attached by its free end to the board keeps the head securely in position. The entire ventral surface of the neck is shaven clean and sterilized by being painted with a saturated solution of picric acid in 70% alcohol. A median longitudinal incision approximately 2.5 cm. long is made, any subcutaneous fat present removed, the sternohyoid and sternothyroid muscles on one side dissected and reflected laterally by means of a small clamp, and the thyroid gland thus exposed. The dissection of the gland from the trachea begins at the posterior end and proceeds anteriorly. Fine, though blunt, forceps are used, and particular care is taken not to injure the laryngeal nerve. The

inferior thyroid blood vessels need not be ligated as the haemorrhage from them is slight and insignificant. It is the anterior end of the thyroid which presents serious difficulties. At this point the gland is applied very closely to the larynx and, to complicate matters further, the superior thyroid blood vessels enter the thyroid gland in proximity of its anterior tip. These latter, when cut, bleed profusely. The operator is therefore confronted with the task of effectively ligating the blood vessels without leaving the slightest thyroid remnant in the knot. This task is usually accomplished by making several ligatures, one below (i.e., anterior to) the other, and by snapping the tie just above the last ligature. The finest silk is used for ligating the thyroid blood vessels. The identical procedure is repeated on the other side, the muscles readjusted and the incision closed with a strong silk of the button-hole twist variety. The wound is sterilized with the alcoholic picric acid solution mentioned above and is covered with 20% celloidin.

When thus treated, the wound heals rapidly and satisfactorily. If careful asepsis is practiced during the operation no infection need be feared. The outcome of the thyroidless, and often also parathyroidless, condition produced is, however, very uncertain. The parathyroids present individual variations in number and position in relation to the thyroids. Usually they are found on the surface of the thyroid gland or embedded within its stroma. Sometimes a parathyroid located on the surface of the thyroid sends one or more projections into the latter's substance. Rarely parathyroid nodules have been found detached from the thyroid, but in its immediate proximity. In the living animal, it is usually impossible to distinguish parathyroid tissue from thyroid, and therefore, the two, unfortunately, cannot be separated. Occasionally, however, an animal is found to have parathyroids which appear coal-black and show, on histological examination, a number of melanophores within their stroma. In such cases at least one parathyroid is carefully dissected from the thyroid and left in situ intact. If the parathyroids in a given animal

have been removed completely along with the thyroids, the animal in question will succumb to tetany usually within 3 days to approximately 3 weeks, depending upon the severity of the calcium deficiency incurred. For prevention of tetany, postoperative administration of soluble calcium was resorted to. Injection of sterile 10% calcium gluconate proved disappointing as the animals subjected to this treatment became extremely irritable, and extensive, infection-inviting necroses formed at the sites of injection. After considerable experimentation and heavy losses in animals, mortality was reduced to a low minimum by oral administration of calcium in the form of powdered calcium lactate mixed with the food (ground grain) and 5% aqueous calcium gluconate added to the drinking water.

Most of the animals thus supplied with calcium survived until February, March or April when they were sacrificed. Only a few of these survivors displayed normal appearance and behavior. The greatest majority of them showed general emaciation, shabbiness of coat, and extreme irritability. Some would go into convulsions characteristic of tetany when seized or otherwise violently excited and would usually recover after 2 to 3 minutes of rest. Several animals, however, instead of losing weight, grew heavy, fat and flabby, became extraordinarily sluggish and cold to touch, and developed a decided inclination toward torpidity. This behavior is particularly interesting since normal ground squirrels did not hibernate at room temperature.

The character and degree of sexual development in both operated and untreated males were examined at intervals of 1 to 2 weeks throughout the experimental period (October to April) by means of manual palpation of the testis, the epididymis and the bulbar gland, and by observations of the scrotal skin for pigmentation. This procedure was deemed necessary, since we did not wish to overlook the possibility that if and when reproductive development did occur it might be followed by sexual regression previous to the date of autopsy.

All experimental animals, immediately after being sacrificed and usually before the blood circulation stopped completely, were perfused through the heart with a 0.0025% aqueous solution of naphthol blue (Meldola) which invariably stained blue all thyroid and parathyroid remnants present in the animal. The neck region was then searched carefully under a dissecting microscope for regenerated thyroid nodules, and all suspicious tissue was fixed in Bouin's fluid as was the entire neck exclusive of the skin and paravertebral region. If, on histological examination, the bits of suspicious tissue recovered proved not to be thyroid, or if no suspicious tissue had been found at autopsy at all, the fixed portion of the neck was embedded in celloidin, sectioned serially, stained in alcoholic haemalum, and examined for presence of thyroid tissue. The absence of the latter in the serial sections of the neck was taken as a final conclusive indication that thyroidectomy had been complete in the given animal.

Histological study of the neck region revealed only eleven absolutely thyroidless males (nine young and two adult at the time of operation), and most of these lacked also any traces of parathyroid tissue in neck levels. Ten additional animals showed exceedingly slight thyroid regeneration (6 mg. or less) and absence of parathyroids, and exhibited unequivocal symptoms of hypothyroidism and hypoparathyroidism. Four other animals possessed as much as 9 to 14 mg. of regenerated thyroid tissue. Only the twenty-five operated animals mentioned in this paragraph attained the qualifications for consideration in the present report as thyroidectomized males, since they alone were the ones that survived long enough to permit autopsy during February, March and April.

Testes, seminal vesicles, prostate gland, Cowper's glands and bulbar glands from the twenty-five experimental and the twenty-one control animals were weighed while fresh and fixed in Bouin's picro-acetic-formol. Smears of fresh epididymis in physiological saline solution were studied microscopically for presence and motility of spermatozoa. The remaining part of epididymis and the ductus deferens were

then fixed without weighing. Six-micra sections of each of the above-mentioned organs from twenty-one operated males were examined microscopically and compared with similar preparations of these reproductive organs from four typical control males killed between February and April. Previous observations by the junior author of reproductive organs from normal males of this species, at all stages of five complete annual sexual cycles, have been of great value in the present investigation.

RESULTS

It should be stressed that all animals were observed and studied by manual palpation at frequent intervals, and that both experimental and control males considered in this report were sacrificed during the time when all of the untreated controls exhibited normal breeding capacity characterized by functional accessory reproductive organs and motile spermatozoa. Some males on the date of operation were immature, while others although somatically adult were in a state of sexual involution (aspermatic testes, non-functional accessories) at the time of operation. Also, it should be recalled that in most cases parathyroid glands were removed along with the thyroids, hence, such animals at autopsy were parathyroidectomized as well as thyroidectomized individuals. An experimental male was not considered to be completely thyroidectomized, however, unless no thyroid tissue was detectable by gross and histological methods following sacrifice.

1. Control males

Testes of untreated animals at autopsy were invariably located in a scrotal position. They contained all elements of the germinal epithelium including spermatozoa. The weight of the testis was variable, since normal animals of this species possess heaviest testes previous to the mating season and smaller gonads at the peak of the breeding period. The weight of the testis for individual controls varied from 1.1260 gm. (February) to 0.3550 gm. (April), but this extreme variability

was not encountered when animals killed during any one month were compared (all control animals except one had a testis weight of 0.51 gm. or greater, and such testes regardless of weight had produced spermatozoa which were motile in physiological saline solution).

Accessory reproductive organs of all untreated males, with one exception, were characteristic of those in normal rutting adults. This exceptional control animal, no. A9, was sacrificed on February 2nd; whereas its testis weighed 1.1260 gm. and had produced spermatozoa, its accessory organs had not reached their maximal development. The most extreme deviations from the average weights (table 1) for accessories of control males were encountered in animals A9 and A17, killed on February 2nd and April 12th, respectively, and the respective weights of their fresh organs in grams were: prostate gland, 0.0978 and 0.3000; seminal vesicles, 0.1840 and 1.2440; bulbar gland, 0.3758 and 0.9064; Cowper's glands, 0.1614 and 0.4480. Histological studies revealed all of these organs, along with epididymis and ductus deferens, from four typical control animals to be characteristic of breeding adults.

2. Operated males with regenerated thyroid tissue

In this group are fourteen animals in which some thyroid tissue was either macroscopically or histologically detected. The amount of regeneration varied a great deal (table 1). Three animals showed no gross but positive histological evidence of thyroid fragments, while eleven had weighable amounts varying from approximately 2.6 mg. to almost complete regeneration of thyroid glands. All these males received calcium in their food and drinking water.

Sexual development in twelve of these fourteen experimental animals paralleled that found in unoperated controls, and in no discernible way did the reproductive organs of these operated animals differ qualitatively from those of untreated males.

Two operated males (A106, A120) differed from the others by failing to attain as great a degree of sexual development at

TABLE 1
Gross and microscopic condition of reproductive organs following thyroidectomy

ANIMAL NO.	OPERATION		DAYS BETWEEN OPERATION AND AUTOPSY	AUTOPSY		AMOUNT OF REGENERATED THYROID TISSUE	WEIGHT OF FRESH REPRODUCTIVE ORGANS IN GRAMS					SPERMATOZOA		AMOUNT OF SCROTAL PIGMENT	HISTOLOGICAL DEVELOPMENT OF ACCESSORIES
	Date	Body weight in grams		Date	Body weight in grams		Testis	Seminal vesicles	Prostate gland	Cowper's glands	Bulbar gland	Testis	Epi- didymis		
<i>Operated males with regenerated thyroid tissue</i>															
A106	Oct. 16	186	198	Mar. 1	158	2	0.2494	0.4325	0.1160	0.1610	0.4750	0	0	3	3
A138	Nov. 27	236	101	Mar. 7	170	2	1.1054	0.5542	0.1852	0.2140	0.4698	4	4	4	3
A118	Oct. 31	216	128	Mar. 7	180	4	0.4477	0.6494	0.1720	0.2320	0.5952	4	4	4	4
A129	Nov. 14	180	128	Mar. 21	156	1	0.5396	0.8489	0.2045	0.2470	0.6686	4	4	4	4
A 52	Nov. 19	196	125	Mar. 23	148	2	0.5400	0.8302	0.2094	0.2612	0.6744	4	2	4	4
A 79	Sep. 22	160	185	Mar. 25	136	2	0.3816	0.6240	0.1545	0.2365	0.5314	1	1	4	3
A135	Nov. 23	200	124	Mar. 26	192	4	0.7682	0.8146	0.1728	0.2520	0.4614	4	4	4	4
A102	Oct. 14	204	165	Mar. 27	172	3	0.5122	0.7740	0.1776	0.2290	0.7317	4	4	4	4
A109	Oct. 17	208	163	Mar. 28	178	4	0.7109	1.0466	0.2570	0.2774	0.7498	4	4	4	4
A142	Dec. 2	242	117	Mar. 29	208	2	0.6882	1.3061	0.2436	0.3564	0.6245	4	4	4	4
A117	Oct. 30	180	153	Mar. 31	162	1	0.4302	0.5738	0.1182	0.1622	0.4124	4	4	4	4
A110	Oct. 17	210	170	Apr. 4	202	1	0.4919	0.6886	0.1821	0.1999	0.6410	4	4	4	4
A136	Nov. 23	200	133	Apr. 4	202	3	0.6460	0.7988	0.2070	0.2972	0.5780	4	4	4	4
A120	Nov. 5	156	175	Apr. 29	116	2	0.2190	0.4342	0.1138	0.1822	0.3404	0	0	4	3
Average		198			170		0.5493	0.7204	0.1795	0.2362	0.5409				
<i>Operated males with no detectable thyroid tissue</i>															
A140	Nov. 29	176	64	Jan. 31	60		0.0702	0.0060	0.0032	0.0068	0.0238	0	0	0	1
A 69 ¹	Aug. 23	188	169	Feb. 7	76		0.2310	0.0114	0.0034	0.0118	0.0358	0	0	1	1
A 93	Oct. 2	168	134	Feb. 13	94		0.1357	0.0170	0.0063	0.0078	0.0413	0	0	1	1
A141	Nov. 29	174	77	Feb. 13	48		0.0794					0	0	1	1
A 87	Sep. 30	148	138	Feb. 14	104		0.2028	0.0159	0.0063	0.0146	0.0312	0	0	0	1
A116	Oct. 30	140	121	Feb. 27	168		0.8895	0.5576	0.1475	0.1630	0.3944	4	4	4	3
A 97	Oct. 9	152	169	Mar. 26	152		0.2909	0.7164	0.1720	0.2790	0.4453	1	1	4	4
A111	Oct. 17	176	162	Mar. 27	192		0.2330	0.7444	0.1456	0.2596	0.6496	1	0	4	4
A 99	Oct. 10	168	199	Apr. 27	200		0.3015	0.0168	0.0100	0.0412	0.0584	1	0	1	1
A126 ¹	Nov. 13	176	169	Apr. 29	116		0.0792	0.0634	0.0178	0.0388	0.1502	0	0	0	1
A123	Nov. 5	176	177	Apr. 30	128		0.0943	0.0110		0.0036	0.0236	0	0	1	1
Average		168			121		0.2461	0.2159	0.0569	0.0826	0.1854				
<i>Unoperated control animals</i>															
Average for 5 animals 12 animals 4 animals				Feb.	229		0.8022	0.5762	0.1561	0.2413	0.5491	4	4	4	4
				Mar.	206		0.6430	0.7537	0.1784	0.2743	0.5910	4	4	4	4
				Apr.	219		0.5846	0.9657	0.2315	0.3450	0.6941	4	4	4	4

Amount of regenerated thyroid tissue found at autopsy expressed in categories from 1 (approximately 2.5 to 5.0 mg.) to 4 (over 10 mg.). Estimated quantity of spermatozoa and scrotal pigment recorded in designations from 0 (none) to 4 (characteristic of breeding males). Estimated histological condition of accessory organs designated in categories from 1 (infantile condition) to 4 (breeding state).
¹ Adult at the time of operation which occurred while reproductive organs were in an involution condition.
² No thyroid tissue macroscopically detectable. Small thyroid fragments were found upon examination of serial sections of paratracheal region.
³ Histological study of reproductive organs made for only four typical animals in this control group.

the time of their autopsy, April 29th and May 1st, as that demonstrated by April control animals (table 1). These two were studied at frequent intervals while alive, and it is certain that breeding activity did not occur previous to the date of sacrifice, although the testis, as determined by palpation, had previously reached a size approximately 40% of the normal maximum in animal A106, and about 50% of the normal maximum in animal A120. Although animal A106 had approximately 5.6 mg. of thyroid tissue and A120 only traces identifiable in serial sections of the neck, their reproductive organs were essentially alike. Testes of each male were abdominal in position and small, weighing less than those from any individual control animal, and they were found to contain no germinal elements more advanced than non-metamorphosing spermatids, i.e., there were no spermatozoa present. Accessory organs of these two males in qualitative and quantitative development resembled those of untreated animals killed in February, and had advanced histologically only to a state definitely below the peak for breeding adults; both grossly and microscopically, organs of the animal having the largest thyroid fragment were slightly more developed than those from the one with a smaller regenerated fragment, although autopsy dates were only 2 days apart.

3. Males with no detectable thyroid tissue

No thyroid tissue was found in eleven experimental animals. Only three of these showed sexual development simulating that found in control males, while the other eight had reproductive organs entirely different from those of untreated animals.

The weight of the testis varied from a value approximately equal to that for untreated males (animal A116) to one only 13% as great as the average for four April controls (A126). Average testicular weight for this entire experimental group was only 34% as great as that for the twenty-one control males.

Microscopical study revealed that testicular development in seven of the operated animals had not progressed beyond the formation of spermatocytes (figs. 1, 2); five of these males were immature at the time of operation, while the other two were adults with involuted reproductive organs on the date of thyroid removal (table 1). The testis from the remaining four experimental males contained spermatids and spermatozoa, the quantity of spermatozoa varying from few (animals A97, A99, A111) to very many (A116); it is interesting to note that these four animals were the only ones in this experimental group that showed no decrease in body weight during the experimental period.

Accessory reproductive organs of such experimental males at autopsy, with three exceptions (A97, A111, A116), were in a non-functional condition and similar to those of normal immature animals, i.e., the developmental degree of accessories at autopsy differed little, if any, from that of the same organs at the time of operation. Thus, the weight of the prostate gland² from untreated controls was approximately twenty-five times greater than the average weight of the prostate for all operated males of this group (excepting A97, A111, A116).

In each of the three exceptional animals, all accessory organs showed high-grade development and functional activity. When judged by both macroscopical and histological studies, accessories of such males were almost as well developed as those from unoperated males. It will be recalled that each of these three males had produced spermatozoa. Animal A99 presented a still different condition at autopsy; its small testes contained a few spermatozoa, but its accessories had not developed beyond an infantile condition.

² The weight of the prostate gland is not influenced by accumulated secretions, since in the case of the prostate they are not stored within the gland in this species. Therefore, the prostate gland serves as a sensitive index of the gross condition of the accessory reproductive organs.

DISCUSSION

Data reported above indicate that an absence of thyroid tissue in male ground squirrels may prevent the normal development and function of reproductive organs. In eight of the eleven operated males (72.7%) revealing neither gross nor microscopic traces of thyroid fragments in neck regions, the entire reproductive system was in a non-breeding condition. Two of these were adults while the other six were immature at the time of operation, therefore thyroid removal apparently prevented sexual development in both adult and immature males. Three males, however, likewise lacking detectable fragments of thyroid tissue did undergo sexual development practically simulating that of unoperated males, and it is obviously possible that such animals may actually have possessed some thyroid tissue which escaped our observation. That this may have been the case is suggested by the fact that all operated males, with detectable thyroid regeneration, exhibited high-grade sexual development, and twelve of the fourteen animals in this lot (85.7%) had produced spermatozoa.

Parathyroids were removed along with the thyroids in most animals, and the ensuing tetany was controlled by calcium feeding. We realize that absence of the parathyroid gland makes it impossible for us to be positive that our results were due entirely to thyroid absence. We believe, however, that our data suggest strongly that the effects noted were due mainly to an absence of the thyroid glands, since operated animals whose tetany was controlled with calcium showed essentially normal reproductive organs when thyroid regeneration had occurred. Confusion due to possible effects of calcium administration was adequately avoided by the fact that our unoperated controls likewise received calcium in their food while living in the same cage with operated males.

A search through relevant literature reveals, in the main, findings agreeing with our results. Thus, it has long been recognized that, in the mammalian male, experimentally produced athyroid or hypothyroid states tend to suppress testicu-

lar development and function. The effect is particularly pronounced in animals thyroidectomized prepuberally and carried to the age of maturity. As early as 1894, Hofmeister observed that most of his rabbits which had been thyroidectomized when 5 weeks to 4 months old and had developed unmistakable symptoms of cachexia, yielded small, underdeveloped, and in some cases aspermatic testicles. Similar results were obtained with lambs and kids by von Eiselsberg (1895) and corroborated on kids by Lanz ('04). Tatum ('13) thyroidectomized immature rabbits 2 to 3 weeks old, weighing 150 to 250 gm. each, and found that the testes of the experimental males at autopsy showed atrophy of the seminiferous tubules, but that the interstitial cells of Leydig appeared perfectly normal in number and structure. Hammett ('22) reported that completely thyroidectomized male rats were capable of fertile mating even with completely thyroidectomized females, although the production of mature spermatozoa in normal quantities was markedly disturbed in the experimental males. Male rats thyroidectomized at 4 to 6 months of age and autopsied 40 to 200 days later were shown by Smelser ('34) to have fewer sperm and smaller accessory reproductive organs than unoperated controls. Differing somewhat from our results are those obtained by Ross ('36) who found that in immature male rats thyroidectomy leads not to suppression of sexual development and reproductive activity, but merely to a delay in the onset of sexual maturity. She also reports that thyroidectomy in mature male rats produced no histological changes in the testis, seminal vesicle, or prostate gland, but that the operation resulted in a slight decrease in the weight of the seminal vesicle and in a frequent loss of the sex interest. It must, however, be borne in mind that in the case of the rat we deal with a constant breeder, whereas in the ground squirrel we have an animal with a strictly seasonal reproductive cycle, and this difference may account for the discrepancy in the results. Most of these findings, then, indicate clearly that while the proper functioning of the thyroid gland

in the constantly breeding males of the forms studied³ may not be essential in the control of reproductive processes, yet somehow it represents a link in the chain of endocrine influences underlying normal sexual development and activity. A study of the effect of thyroidectomy upon the reproductive system in an annually-breeding wild mammal has, to the writers' knowledge, never been attempted before; our results seem particularly stimulating, since we find that the thyroid glands appear to be essential for normal sexual development and function in males of a wild mammal with an annual period of rut.

We wish to stress the fact that our data do not definitely establish the precise relation between the thyroid glands and the reproductive system of the male ground squirrel. We do feel, however, that the data presented have suggestive value. Attempts at solution of the question by several experimental approaches are now being made by the senior author.

SUMMARY AND CONCLUSIONS

Thyroidectomy, usually accompanied also by parathyroidectomy, was performed on 120 male ground squirrels during the period of sexual quiescence (October to November) to determine the effect of the operation on the reproductive system. Only twenty-five of these that survived until the time when unoperated laboratory controls exhibited breeding capacity (February to April), qualified for consideration in this paper. Genital organs of both operated and control males were studied by observation and manual palpation at intervals of approximately 2 weeks during the experimental period, and all reproductive organs were weighed at autopsy and prepared for microscopical study.

Gross inspection and microscopical study of serial sections of the neck furnished decisive criteria for the presence or absence of regenerated thyroid remnants.

³ Carnivores do not lend themselves to successful experimentation with operative cretinism on account of the usually fatal outcome of complete thyroparathyroidectomy in these species.

All twenty-one controls attained during the breeding period a high degree of sexual development. Eight of eleven experimental males (72.7%) possessing no detectable thyroid tissue did not develop, with regard to either spermatogenesis or testis hormone production, beyond an essentially infantile condition (seven cases; five young and two adult at the time of operation), or reached a state of very low sexual activity (one case). The other three attained breeding capacity, and their reproductive organs, both in germ cell production and hormone secretion by the testis, resembled, in the main, those of controls; it is possible that these possessed some thyroid tissue that escaped out detection.

Fourteen operated males with detectable thyroid regeneration underwent varying degrees of sexual development which approximated that of control animals.

Our data suggest strongly that the thyroid glands are necessary for development and function of male reproductive organs in a wild mammal (both immature and adult) which normally has a short annual period of breeding capacity and rut. Determination of the precise relation of the thyroid gland to the annual sexual cycle must await the results of additional experiments now being performed by the writers.

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PLATE

PLATE 1

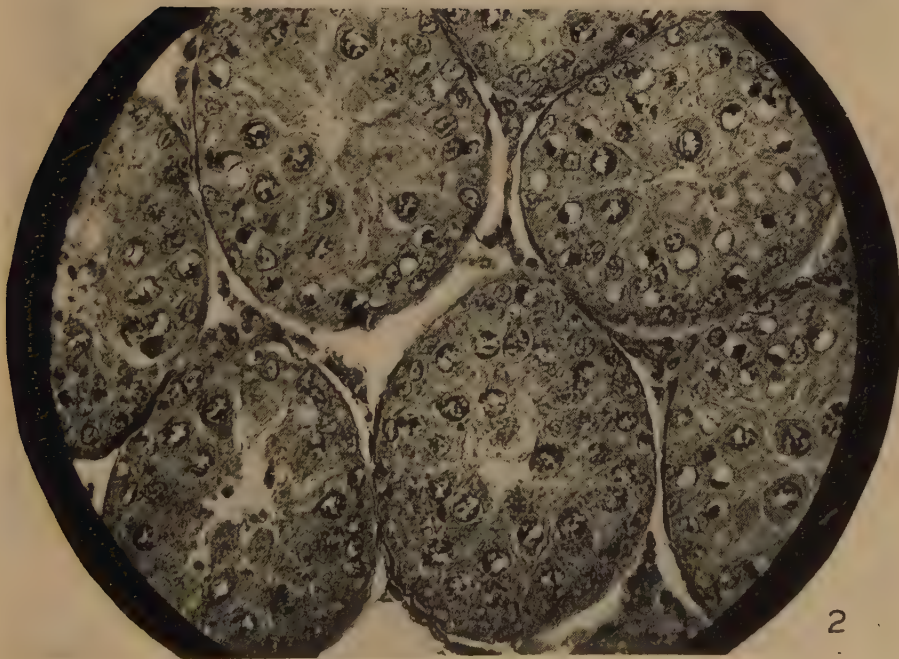
EXPLANATION OF FIGURES

1 Portion of the testis from an unoperated control animal which was killed 2-15-36. Weight of the fresh testis was 0.8562 gm. Note the spermatozoa in the seminiferous tubules. Section cut at 6μ . $\times 305$.

2 Typical part of the testis from thyroidectomized male A69 which was autopsied 2-7-36, $5\frac{1}{2}$ months after thyroidectomy. The weight of the fresh testis was 0.2310 gm. Note the absence of spermatozoa in the seminiferous tubules. This animal was an adult showing normal involution of reproductive organs on the date of operation, 8-23-35; it showed no regenerated thyroid tissue in serial sections of the neck, and it failed to undergo normal sexual development. Section cut at 6μ . $\times 305$.



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2

TRANSFORMATION OF LYMPHOCYTES INTO GRANULOCYTES IN VITRO ¹

WILLIAM BLOOM

Department of Anatomy, The University of Chicago

TWO PLATES (SIX FIGURES)

This report is a description of the conditions under which the transformation of lymphocytes into pseudo-eosinophil leukocytes occurs in cultures of rabbit lymph.

The literature on ectopic myelopoiesis in vitro is meager. In 1917 and 1923, Maximow reported that he had found eosinophil and pseudo-eosinophil myelocytes and a few megakaryocytes developing from large and small lymphocytes in portions of rabbit lymph nodes cultured for 4 days in plasma and bone marrow extract. Shiomi ('25) failed to obtain myelocytes in his attempt to repeat this experiment. Latta and Johnson ('34) noted the development of eosinophil myelocytes from lymphocytes in cultures of rat lymph nodes. Maximow's conclusion that the myeloid elements developed from lymphocytes was criticized by Michels ('23) who believed that they might have come from the reticular cells in those cultures.

MATERIALS AND METHODS

It has been a common observation that most of the lymphocytes in the migration zone of cultures of lymphatic tissue die after a day or two in vitro, whereas those within the explant, and particularly those between the explant and the coverslip, may remain alive for several weeks if the cultures are washed every few days and supplied with fresh nutritive media. It had previously been found that, depending on the medium

¹ This investigation was aided by a grant from the Rockefeller Foundation to The University of Chicago.

used, rabbit lymphocytes of the thoracic duct, when explanted alone, either die shortly or change into macrophages.

Accordingly, in the attempt to determine whether rabbit lymphocytes have myelopoietic potencies, it was necessary to find a means of keeping the lymphocytes alive and unchanged so that various other stimuli might have the opportunity of acting on them for several days. When rabbit lymph mixed with equal volumes of rabbit embryonic extract was explanted with rabbit connective tissue, the lymphocytes turned into macrophages. When rabbit bone marrow extract was substituted for the embryonic extract in this experiment, the lymphocytes remained alive but did not change into either macrophages or myelocytes. Attempts to replace the living connective tissue with dead connective tissue or other inert materials as cellophane, gelatin, glass wool, etc., were failures, as the lymphocytes died in these media.

In view of the intense eosinophilia which develops in animals infected with animal parasites or injected with extracts of them, the effect of extracts of parasites was tried on lymph cultures. To this end, a fresh extract of porcine *Ascaris lumbricoides* 1:200 in Tyrode solution was explanted with the lymphocytes, bone marrow extract, and connective tissue. Myelocytes did not develop in these cultures.

Since eosinophil leukocytes may develop during the process of immunization (see Taliaferro, '29, p. 628), the next experiment tried was to obtain lymph from an immunized rabbit and add the antigen to the cultures. Accordingly lymph was cultured from two rabbits previously infected (and thus immunized) with trichinous meat by Dr. C. Huff of the Department of Bacteriology and Parasitology of The University of Chicago. These rabbits developed trichinosis and the lymph was taken 50 and 60 days after the infection of the animals. This lymph was explanted with bone marrow extract, connective tissue, and living free and encapsulated trichina worms. The lymphocytes in these cultures developed into macrophages but not into myelocytes.

When the following procedure was tried, the lymphocytes developed into granular leukocytes. It consisted in immunizing rabbits from which the lymph was to be taken with four subcutaneous injections, at 2-day intervals, of 2 cc. of fresh ascaris extract, 1:200, in Tyrode solution. Two weeks after the last injection lymph was obtained from the thoracic duct. When such lymph, together with equal amounts of ascaris and bone marrow extracts, was cultured with the connective tissue of a normal rabbit or chicken, it was found in five experiments (i.e., in cultures of lymph from each of five rabbits) that numbers of heterophil (pseudoeosinophil) myelocytes and leukocytes developed in some of the cultures after 5 to 7 days in vitro. Myelocytes failed to develop in lymph cultures from two other similarly treated animals and in one series of cultures of similar lymph in which the fresh ascaris extract was replaced by the polysaccharide of ascaris which Campbell ('36) has shown to be actively antigenic. Although rabbit connective tissue was used in the majority of the experiments, rabbit myelocytes also developed when rabbit lymphocytes were explanted with chicken connective tissue. These cultures are important because of their value in excluding several possible sources of error in the interpretation of the results of these experiments, for the heterophil granules of the rabbit are easily distinguished from those of the chicken. In every series a large number of control cultures was made of connective tissue without lymph but with bone marrow extract alone or with ascaris extract. Myelocytes did not develop in these cultures.

Method of obtaining rabbit lymph for culture. The lymph was obtained from rabbits, anesthetized with ether, by laying bare the thoracic duct and placing a ligature around the vessel just before it emptied into the jugular vein. The duct was difficult to find in all of these animals because, as previous work had demonstrated, it was necessary to starve the rabbits for 48 hours to remove the large amount of fat present in the thoracic duct after digestion. Shortly after the ligature was applied, the duct became greatly distended. It was then

incised and the lymph allowed to flow out and cover all of the surrounding muscles, blood vessels and connective tissue. This lymph soon clotted and successive drops of lymph emerging from the duct also clotted, so that after a few minutes the possibility of contaminating the lymph so obtained by connective tissue or blood cells was excluded. This technic was developed by Kindwall ('27), and in my experience it has been just as satisfactory a method of obtaining pure lymph as the much more tedious and often unsuccessful direct cannulation of the duct. After the area surrounding the duct was covered with a clot of lymph, a very fine pipette was inserted into the incision in the duct and the lymph aspirated. As the animal continued to breathe, more and more lymph came out of the duct. The collection of the lymph and the subsequent procedures in making the cultures were carried out aseptically. Many portions of lymph were taken for control purposes. After sufficient lymph was obtained for the cultures, the rabbits were killed. The thoracic duct and parts of all the viscera were fixed for microscopic examination.

Culture technic. All of the cultures were made by Maximow's double coverslip method with heparinized rabbit plasma and various extracts. The living cultures were studied with and without supravital staining with neutral red and Janus green, alone or in combination. The cultures were washed in Tyrode solution and given fresh extracts every 2 days. One hundred and fifty to 200 cultures were made of each series and these were fixed at close intervals with Zenker-formol, embedded in nitrocellulose, serially sectioned, and stained in hematoxylin-eosin-azure II.

Bone marrow extract was made by suspending the bone marrow of two femurs of a young rabbit in six parts of Tyrode solution. This was centrifuged at 3000 revolutions for 8 minutes. The clear extract was then pipetted and rapidly frozen and thawed six times or filtered through a Seitz filter to exclude the possibility of carrying living myeloid cells into the cultures with the bone marrow extract. In the early experiments duplicate sets of cultures were prepared with

fresh bone marrow extract made apparently cell-free by centrifugation and with a similar extract which was then frozen and thawed repeatedly. The frozen extracts seemed as effective as the non-frozen ones in producing myelocytes.

Ascaris extract was prepared by suspending 1 gm. of fresh, minced, porcine *Ascaris lumbricoides* in 200 cc. of Tyrode solution overnight at 6°C., and then sterilized by passage through a Berkefeld filter. The ascaris was kindly furnished by Dr. D. H. Campbell of the Department of Bacteriology and Parasitology of The University of Chicago.

The first experiments were made by injecting the whole lymph into the connective tissue of a freshly killed rabbit or chicken and explanting minced portions of the bleb thus produced. In later experiments fresh lymph, or a suspension of lymphocytes obtained by centrifuging heparinized lymph, was injected into cultures of connective tissue.

Cells of the rabbit thoracic duct lymph. The cells of the lymph of the thoracic duct of the rabbit are almost exclusively large, medium-sized and small lymphocytes, according to Thorne and Evans ('22), Simpson ('22), Kindwall ('27), and Bloom ('28). In addition, rabbit lymph always contains fair numbers of erythrocytes and an occasional monocyte and eosinophil leukocyte. With the exception of one rabbit which had a very intense experimentally induced myelopoiesis in practically all of the organs, I have never seen a pseudo-eosinophil leukocyte or any type of myelocyte or erythroblast in the thoracic duct lymph of any rabbit. These observations were made with supravital, dry, and moist fixed smears of the lymph, serial sections of the thoracic duct fixed between two ligatures, and studies of the early stages of lymph cultures in the living condition and after fixation and staining.

The lymphocytes in rabbit lymph are quite typical of those found in the lymphatic tissue of the same animal; the small lymphocytes usually form about 80% of the cells present. The large lymphocytes, which are the cells probably confused by some of the older authors with large mononuclears (i.e., monocytes), have a large nucleus with heavy chromatin

particles and one or several very large acidophil nucleoli. The basophilia of the cytoplasm is quite intense when stained with eosin-azure II. In the living condition, one to three or four fat droplets may be seen in these large lymphocytes and practically none of them contains more than five or six neutral red stainable bodies. The mitochondria, as shown by Janus green as well as by the usual mitochondrial technics after fixation and staining, are fairly large and relatively few in number in most of the cells. It may be pointed out that the development of the explanted lymphocytes of the lymph into monocyte-like cells, macrophages and fibroblast-like cells has been described (Bloom, '27, '28). The correctness of these observations has been corroborated by Bergel ('30), and denied by Seemann ('30) on the basis of one experiment. Ehrich ('34) cultured the lymph of popliteal lymph nodes of rabbits and found that while the small lymphocytes did not hypertrophy, the larger ones developed into macrophages. Because of this transformation he concluded that the large cells of the lymph were monoblasts and not lymphoblasts. Murakami ('36) cultured the lymph from the popliteal lymphatics of seven rabbits. The lymphocytes died in all of the cultures, save those of one series in which they apparently developed into fibroblasts.

Tissues of the immunized rabbits. Sections through the tissues of rabbits immunized with ascaris extract from which the lymph was obtained show no changes from the normal except the bone marrow which is depleted and contains but small foci of hematopoiesis. The red pulp of the spleen shows a low grade ectopic myelopoiesis with heterophil and eosinophil myelocytes developing mainly from hemocytoblasts and possibly to a small extent from reticular cells. Some of the lymph nodules of the spleen contain reaction centers; a few atypical nodules have centers composed of small lymphocytes with much larger lymphocytes (hemocytoblasts) at their peripheries. The reticular cells throughout the spleen are quite prominent. The lymph nodes seem normal except for an unusual prominence of the reticular cells. Serial sections through the thoracic ducts of the ascaris immunized rabbits

show the cells to be almost exclusively lymphocytes of various sizes. After examining many serial sections, two small macrophages are found among many thousands of lymphocytes.

The rabbit and chicken connective tissues with which the lymph was explanted are normal in appearance.

OBSERVATIONS

Control cultures of connective tissue. The results obtained with the control cultures of adult rabbit and chicken subcutaneous connective tissue explanted with various extracts may be described briefly, for they did not differ from the recorded observations on cultures of this tissue in the usual plasma and embryonic or splenic extract media (Maximow, '16; M. v. Möllendorff, '29; Bloom, '31).

During the first 10 hours practically all the cells within the explant round up and suggest macrophages of various sizes. At this time there are practically no cells which have the appearance of fibroblasts as these cells are usually described in the normal loose connective tissue. After 10 hours, however, some of the rounded cells become elongated and assume a typical fibroblast-like appearance.

Mitoses are not seen for the first 2 days in such cultures and there is usually no migration from them. After this time, however, fair numbers of fibroblast-like cells begin to migrate into the surrounding medium in the rabbit connective tissue cultures, so that after 5 or 6 days the explant is usually surrounded by a thick corona of spindle cells interspersed with a few macrophages. This occurs in all of the cultures of rabbit connective tissue irrespective of the type of extract present—that is, embryonic extract, bone marrow extract, or bone marrow extract plus ascaris extract.

Some of the connective tissue cultures contains bits of striated muscle, portions of nerve, in many cases small lengths of arteries, veins or capillaries, and occasionally groups of fat cells. None of these structures shows anything of interest affecting the present investigation. The endothelial cells of the smaller blood vessels grow in the cultures and frequently

extend for long distances. Their cytoplasm stains slightly more purple than that of the surrounding fibroblasts. In no instance, however, is there any difficulty in recognizing these as endothelial cells, and neither they nor the perivascular cells can be mistaken for lymphocytes. Moreover, as will be described, myelocytes developed only in those connective tissue cultures into which lymph had been injected, and several of the successful cultures did not contain any blood vessels.

The control cultures of adult chicken connective tissue differ from the control rabbit cultures in that the chicken fibroblasts do not thrive in the rabbit plasma and extract. In these cultures there is little increase in the number of fibroblasts and after 4 or 5 days the chicken cells become filled with fat droplets and many of them become vacuolated and die. Hoeppli ('35) found that ascaris extract is unfavorable for growth of chicken chondroblasts *in vitro*. This extract did not harm the rabbit cultures described here.

CULTURES OF LYMPH FROM RABBITS IMMUNIZED WITH ASCARIS

Living cultures. As soon as the cultures are made they are examined, and in practically all instances, dense masses of lymphocytes are seen within and about the bits of loose connective tissue. These cultures thus present a striking contrast to the cultures of connective tissue alone. After some hours *in vitro*, many of the lymphocytes begin to migrate from the zone of the connective tissue; in some cultures at the end of 24 hours this migration is as extensive as in a lymph node culture. The location of the groups of lymphocytes within a given culture is noted and they are examined at least twice a day.

Except for their ameboid movement, no changes are seen in the lymphocytes for the first 3 or 4 days. After 4 or 5 days, however, the living lymphocytes are often definitely larger than on the previous days. When stained with Janus green many of the larger lymphocytes are seen to contain more numerous and smaller mitochondria. The lymphocytes contain very little neutral red stainable material at this time. Their nuclei

are quite large and the nucleoli prominent. In many areas in the living cultures these groups of cells resemble the hemocyto-blasts found in supravital preparations of rabbit bone marrow. These changes in the lymphocytes are found in cultures of every series made from the lymph of the ascaris immunized rabbits and from those infected with trichina. In five of the seven series made with lymphocytes from the ascaris immunized rabbits, but not in either of the series made from the trichina infected rabbits, there is a further development of these cells into myelocytes. This change occurs when the lymphocytes are explanted with either rabbit or chicken connective tissue.

Sometimes during the fifth but more frequently on the sixth day in vitro, many of these large and small lymphocytes are found to contain varying numbers of small granules which stain a faint pink with neutral red. These cells are identical in appearance with the heterophil (special or pseudoeosinophil) myelocytes in supravital stained smears of rabbit bone marrow. Some of the lymphocytes have greatly increased numbers of neutral red vacuoles. These are often grouped in one part of the cytoplasm. Such cells are interpreted as transitions between lymphocytes and monocytes. In addition, typical monocytes are also present in the older cultures.

It is impossible to discriminate very early myelocytes from very early monocytes in these cultures with supravital neutral red stain. During the sixth and especially the seventh and eighth days typical heterophil leukocytes are seen singly or in groups. They are easily recognized by their characteristic lobated nucleus, granules, and movement.

In no case in the living cultures is there any difficulty in separating the lymphocytes from fibroblasts and macrophages.

SECTIONS OF CULTURES

In fixed and stained preparations of these cultures, the most striking change in the first 2 days is the increased basophilia of the perivascular cells. None of them shows any other cytoplasmic characteristics or any nuclear structure differing from those typical of fibroblasts; they have relatively small in-

distinct nucleoli and dust-like chromatin particles. There are no transition forms between them and the lymphocytes.

Most of the lymphocytes are highly ameboid and for the first 4 days *in vitro* show approximately the same proportions of large, medium-sized, and small cells as in the explanted lymph. Those lymphocytes which have moved into the migration zone begin to die after the third day *in vitro*, whereas those within the mother piece are usually very well preserved.

Beginning with the fifth day there is marked hypertrophy of most of the lymphocytes. At this time in some areas, most of them are of the large lymphocyte type and are sharply separated from the surrounding fibroblasts and the occasional macrophages. Such areas are highly suggestive of beginning hematopoietic foci in the rabbit embryo. The large lymphocytes are quite typical, with an intense basophilia of the cytoplasm and very large acidophil nucleoli. In practically all areas, however, small, apparently unchanged lymphocytes can also be found.

In a few instances during the fifth day, but much more frequently on the sixth, many of the lymphocytes contain red or reddish-purple stained granules in their cytoplasm. In the small lymphocytes the granules range from two or three to several dozen to a cell—often completely filling the cytoplasm. The nucleus of such cells, however, is identical with that of the surrounding, unchanged small lymphocytes. In some of the larger lymphocytes the first granules appear as scattered red or purple dots in the basophil cytoplasm, but in most of them the myelocyte granules are grouped in one part of the cytoplasm. In those larger lymphocytes in which numerous granules develop, the cytoplasm loses practically all of its basophilia.

These granules in the myelocytes are morphologically identical with those which occur wherever heterophil (pseudo-eosinophil) myelocytes are formed in the rabbit. In the youngest forms, the granules often stain purple, while in the older forms they are bright red. The granules of these newly developed myelocytes are of quite constant size not only in each

cell but in all cells containing them. They do not resemble the large, dull, very irregularly-sized brownish-red granules which appear in the fibroblasts when cultures have not been washed off sufficiently often; the degeneration granules vary in size from spherules the size of an eosinophil granule to some three or four times as large, and granules of different sizes occur in the same cell. Mitoses are found in a few myelocytes after the sixth day. The myelocytes are usually in groups but in many cultures they are scattered diffusely throughout the mother piece of the culture and, occasionally, in the migration zone close to the explant. In a few 8-day cultures the myelocytes have extended far out into the surrounding medium.

All transitions are present between cells which are typical lymphocytes to similar cells with few or many specific granules. These again are connected by an intimate series of transitions to myelocytes with deeply indented nuclei, and these in turn with mature pseudoeosinophil leukocytes with lobated nuclei. Even in the highly ameboid cells the lobated character of the nucleus is easily discernible.

The number of myelocytes or leukocytes in given cultures shows great variations. In some of them there are only a few myelocytes while in others they are more numerous. In one culture the myelocytes and leukocytes outnumbered the lymphocytes by about 15 to 1. Figures 3 and 4 are drawn from a culture which contains several hundred leukocytes and myelocytes, while the cells of figure 6 are drawn from several cultures, each of which contains about forty myelocytes. In the lymph and chicken connective tissue cultures the myelocytes are all very young. Metamyelocytes and mature leukocytes have not been found. These cultures also contain enormous numbers of dead or dying lymphocytes. The failure of the lymphocytes to develop beyond the myelocyte stage in these cultures is perhaps attributable to the fact that the chicken fibroblasts die in great numbers and probably liberate substances toxic to the rabbit lymphocytes.

Occasionally, the myelocytes and lymphocytes just beneath the coverslip are so flattened that the typical lymphocyte

nucleus is greatly distorted. The attraction sphere becomes quite prominent in many of the larger lymphocytes at about the time at which the first myelocyte granules appear. These cells, however, do not look like plasma cells but are highly suggestive of the hemocytoblasts pictured by Maximow ('09) in the yolk sac of the rabbit embryo. In several cultures after 5 to 6 days *in vitro*, there are a few small collections of plasma cells and transitions between lymphocytes and plasma cells and between lymphocytes and monocytes.

None of the cultures in any of the series contains erythroblasts or megakaryocytes. Cultures were not fixed in alcohol and stained for basophil myelocytes as cells of this type were not seen in any culture stained supravitaly with neutral red.

DISCUSSION

When lymph of the thoracic duct of a rabbit, which had received four injections of ascaris extracts 2 weeks earlier, is explanted with fresh ascaris and bone marrow extracts and living connective tissue of a normal rabbit, heterophil (special or pseudoeosinophil) myelocytes and leukocytes develop in 5 to 7 days from the various-sized explanted lymphocytes. A similar transformation of lymphocytes into myelocytes takes place if chicken connective tissue is substituted for the rabbit connective tissue in this experiment.

In all of the experiments many of the lymphocytes explanted became larger and developed greater numbers of mitochondria after 4 or 5 days *in vitro*; these cultures began to look like the site of a new area of hematopoiesis in the embryo. In five series of cultures the lymphocytes developed into heterophil myelocytes and leukocytes.

The fate of each type of cell in the explanted connective tissue—fibroblast, macrophage, amoeboid wandering cell, perivascular cell, endothelium—has been followed in detail. In none of the control cultures or of the lymph-containing cultures was there any evidence of any of these connective tissue or vascular cells developing into either lymphocytes (hemocytoblasts) or granular leukocytes.

Ascaris extract plus normal lymph had no myelocyte-forming effect nor did lymph from an ascaris immunized rabbit plus ascaris extract. But when lymphocytes from an immunized rabbit are cultured with connective tissue in ascaris extract and bone marrow extract, they develop into heterophil leukocytes.

In reaching the conclusion that the myelocytes and leukocytes developed from the lymphocytes in these cultures, it is necessary to establish: a) That the myelocytes could not have been developed from the bone marrow extract or plasma or from the connective tissue, and b) that they were not present in the explanted lymph. These possible sources of error have been excluded for the following reasons:

1. Only one of the positive experiments was made with unfrozen or unfiltered bone marrow extract.

2. The absence of living or dead megakaryocytes and erythroblasts in these cultures showed that the bone marrow extract was not contaminated with myeloid cells.

3. If myelocytes had been explanted with the bone marrow extract, plasma, lymph or connective tissue, they should have been present in the control cultures or the lymph-containing cultures earlier than the sixth day—and they were not.

4. Myelocytes were not present in the explanted connective tissue and did not develop from its fibroblasts, macrophages, or perivascular cells, for transitional forms were not found between the fibroblasts or macrophages or perivascular cells and the myelocytes in any of the cultures. Further, myelocytes developed in some of the lymph and connective tissue cultures which did not contain blood vessels. The rabbit lymph and chicken connective tissue cultures offer the strongest proof for this point, because in them the myelocytes had granules characteristic of the rabbit and not of chicken heterophil myelocytes.

5. The presence of heterophil myelocytes or leukocytes in the thoracic duct of a normal rabbit has not been reported, although eosinophil leukocytes do occur and were occasionally present in the lymph cultured for these experiments.

In addition to the reasons given above, which are mainly of negative character, there are the positive observations that the lymphocytes were followed from day to day and it was found that some of them became larger and developed more numerous mitochondria before changing into myelocytes. Further, the histological analysis of the cultures shows that the myelocytes in these cultures developed from large, medium-sized and small lymphocytes. Some of the stages in this process have been figured in the accompanying plates.

It was anticipated that the ascaris extract would call forth eosinophil leukocytes. As these did not develop and as heterophil leukocytes did, it would seem that the immunization process has made the lymphocytes able to respond myelopoietically to the bone marrow extract in the presence of more ascaris extract. But this does not explain why, of all the myeloid cell types, only heterophil leukocytes appeared.

Many criteria have been advanced for the morphological separation of myeloblasts from lymphocytes. But none of these has survived the test of experiment. Thus, it has been abundantly proved that myeloblasts and large lymphocytes may have the same number of nucleoli; they are both oxidase negative; they may have the same appearance with supravital neutral red and Janus green staining. This is admitted by Sabin ('32, '36) who agrees that the separation of the two can often be made only on the basis of their progeny. They may have identical nuclear and cytoplasmic structure in dry smears, as is evidenced by the inability of the clinical hematologists to distinguish early myeloblastic from lymphoblastic leukemias, which they call stem cell leukemias; it is only the presence of more mature cells which gives the clue to the direction in which the stem cells are developing.

The objection may be raised that among the lymphocytes of the thoracic duct of the rabbit are occult myeloblasts or other cells which are identical with lymphocytes in appearance but differ from them because they can develop into myelocytes. The opinion that these cells in the thoracic duct are myeloblasts and not lymphocytes merely because they turned into

myelocytes would be a misinterpretation of the whole body of hematological observation and controversy. For the differences in opinion in the past have been based primarily on the presence or absence of reputed morphological differences between the various stem cells. The demonstration in these experiments of myeloid potencies in cells which morphologically and histogenetically are admitted by all to be lymphocytes allows but two interpretations: either a) the lymphocytes have myeloid potencies, or b) myeloblasts which are morphologically indistinguishable from lymphocytes are formed in the lymphatic tissue and enter the circulation by way of the thoracic duct. Until objective criteria are presented for the discrimination of these 'myeloblasts' from the thoracic duct lymphocytes, the latter must be considered to be actual hemocytoblasts with the potencies of the stem cell of the myeloid tissue. Any other interpretation, it seems to me, is merely an attempt to distort facts to fit particular hematological theories.

The transformation of lymphocytes of the thoracic duct, and implicitly of the circulating blood, into myelocytes is evidence in favor of the thesis that the free stem cells of the various hematopoietic tissues have the same potencies for progressive development and that particular lines of differentiation result from definite environmental stimuli which, normally, are different in the several blood cell forming tissues. Most of the work on ectopic myelopoiesis in the body indicates that the various-sized lymphocytes have myelopoietic potencies. The experiments reported here offer corroboration of the correctness of this conclusion. In view of the frequency with which discussions of the presence or absence of myelopoietic potencies in lymphocytes have appeared, it is only necessary to refer the reader to the conflicting expositions of the subject by Dominici ('01), Weidenreich ('11), Pappenheim ('19), Maximow ('27), Naegeli ('31) Ferrata ('34), Doan and Wiseman ('34), Sabin ('36), Downey ('37), and Bloom ('37).

SUMMARY

The various-sized lymphocytes of the rabbits' thoracic duct have been shown to develop into heterophil (pseudoeosinophil) leukocytes in tissue culture.

This occurred after 5 to 7 days in vitro when the thoracic duct lymph of rabbits previously immunized with ascaris extract was explanted with ascaris extract plus cell-free rabbit bone marrow extract and connect tissue of a normal rabbit or chicken. This experiment has been performed successfully in lymph cultures from five rabbits and has failed in those from two others.

Heterophil myelocytes did not develop in a) control connective tissue cultures treated as above except that lymphocytes were not added, or b) in cultures of normal rabbit lymph explanted with connective tissue and bone marrow extract alone or with ascaris extract, or c) in connective tissue explanted with lymph of trichinous rabbits and bone marrow extract with or without the addition of living trichina worms.

The development of rabbit myelocytes in the cultures of rabbit lymphocytes and chicken connective tissue excludes the possibility of a connective tissue cell origin of the myelocytes in those cultures.

The use of filtered or repeatedly frozen and thawed bone marrow extract prevented the cultures from being contaminated with myeloid cells through the extract.

Heterophil myelocytes were not found in any portion of rabbit lymph and were not found in any culture before 5 days in vitro.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

All figures drawn by Miss E. Bohlman with camera lucida from Zenker-formol fixed tissues stained with hematoxylin-eosin-azure II. $\times 1490$.

1 Section through the edge of the thoracic duct of one of the ascaris-immunized rabbits whose lymph was cultured. The cells in the lumen are various-sized lymphocytes. *a*, partially cut cells; *b*, a dead small lymphocyte.

2 Section through group of various-sized lymphocytes in a 7-day culture of ascaris-immunized rabbit lymph and chicken connective tissue. Many of the lymphocytes are larger than those of the explanted lymph. *a*, dead lymphocytes.

3 Lymphocytes and very early myelocytes from 7-day culture of ascaris-immunized rabbit lymph and chicken connective tissue. *a*, medium-sized lymphocytes; *b*, myelocytes developing from small lymphocytes; *c*, myelocyte developing from medium-sized lymphocyte; *d*, small lymphocyte; *e*, medium-sized lymphocyte similar to *c* except for absence of specific granules; *f*, a somewhat larger lymphocyte.

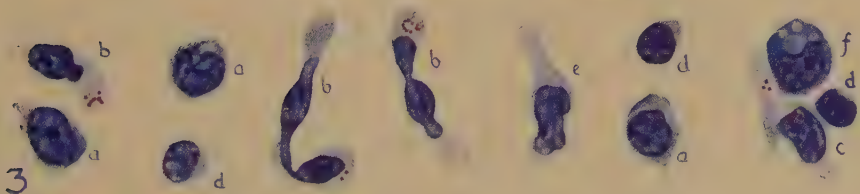
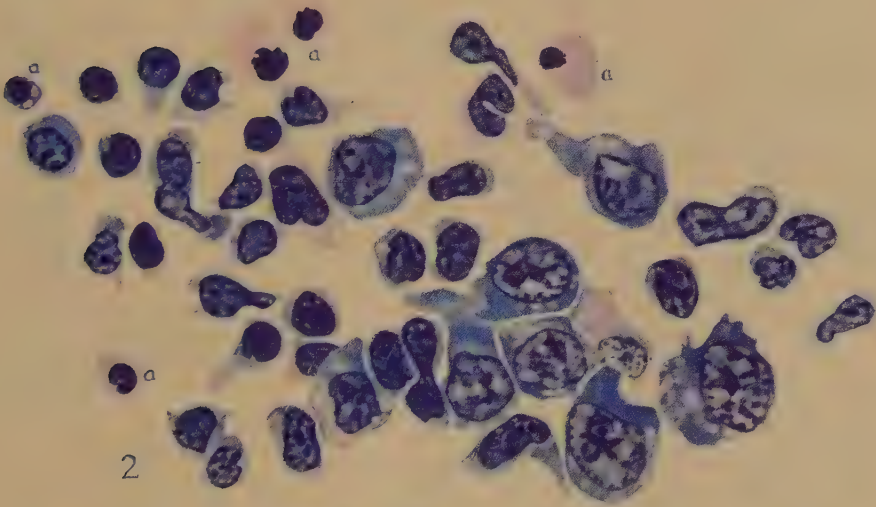
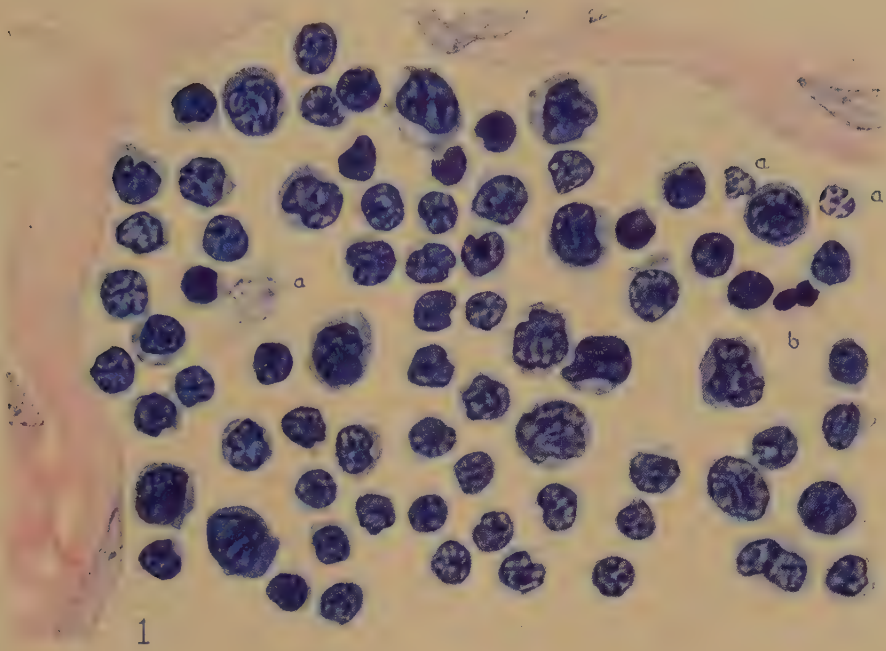
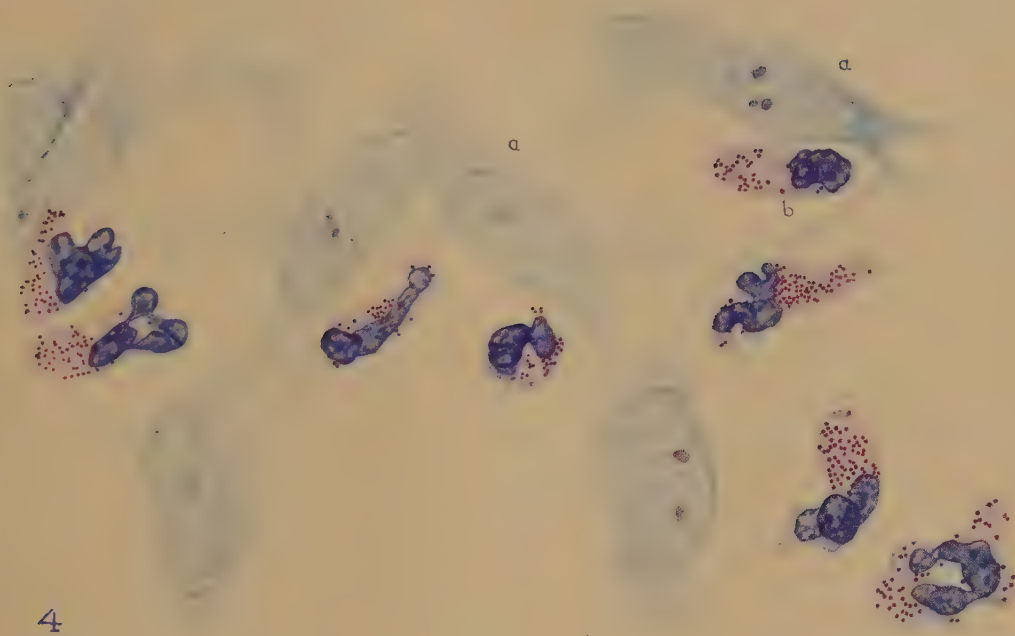


PLATE 2

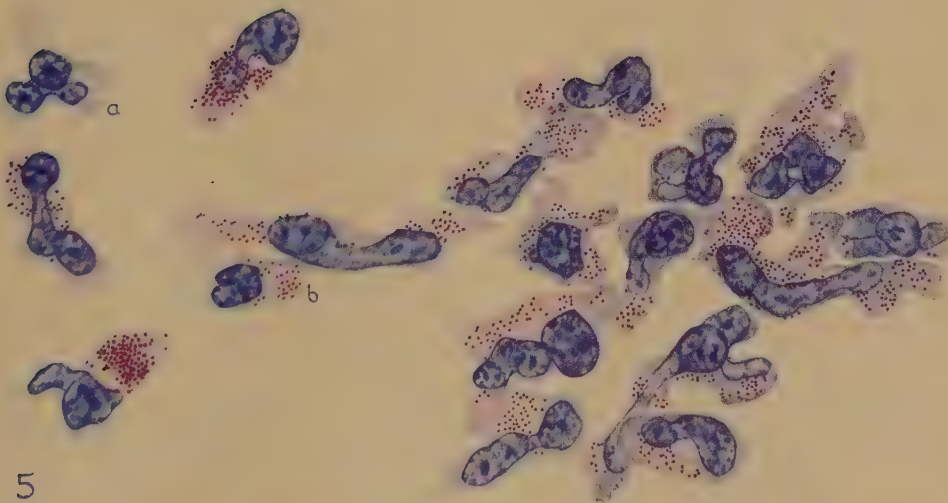
EXPLANATION OF FIGURES

4 and 5 Sections through edge of migration zone of 7-day culture of ascaris-immunized rabbit lymph and rabbit connective tissue. The large fibroblast-like cells (*4 a*) convey an idea of the relative size of the numerous ameboid heterophil myelocytes and leukocytes in the section. The cells were close to the coverslip and greatly flattened. *5 a*, ameboid lymphocyte; cells *4 b* and *5 b* are relatively early myelocytes which still have lymphocytic nuclei.

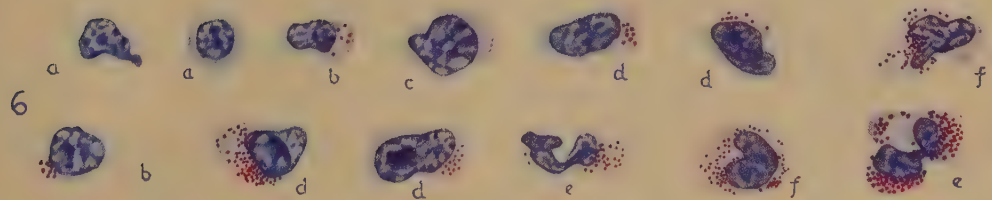
6 Cells from 6- and 7-day cultures of ascaris-immunized rabbit lymph and rabbit connective tissue. *a*, unchanged smaller lymphocytes; *b*, early myelocyte developing from a small lymphocyte; *c*, unchanged larger lymphocyte; *d*, myelocytes developing from larger lymphocytes; *e*, metamyelocyte with few granules; *f*, myelocytes.



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BOOK REVIEW

RECENT ADVANCES IN CYTOLOGY. By C. D. Darlington.
Second edition. Philadelphia. P. Blakiston's Son and Co., 1937.
xvi + 671 pp.

The reader who turns to 'Recent Advances in Cytology' with the hope of finding any extended discussion of cytoplasmic features will be disappointed. Mitochondria are mentioned once, the Golgi apparatus not at all. Of the structures beyond the nuclear membrane only the centrosome receives extended consideration. There are, it is evident, specialists even among cytologists, and this treatise is for those who are interested in the structure and behavior of chromosomes. But though the field covered is in one sense narrow it is in another sense broad, for there is a remarkable consistency in the phenomena of chromosomal behavior throughout a wide range of forms from protozoa to vertebrates, and the impressive body of basic information that cytologists have accumulated is in some measure fundamental to all other phases of biology.

The book is not one to be picked up casually and read of an evening to see what the cytologists have been doing. The style is at times difficult and the reader may need paper and pencil to clarify some of the numerous diagrams or to adapt them to his own mode of thinking. Most readers will profit by having at hand four lengths of differently colored twine to represent chromatids (tetrads), with properly spaced knots which can serve as chromomeres. Scissors and mending material will also prove useful in elucidating some of the relations in inversions and chiasmata ('xta' in the diagrams). In short, one should approach this book much as he would a volume on stereochemistry.

During the past 10 years Darlington has contributed some fifty or more significant papers on cytogenetics and has achieved a position among the most original and stimulating workers in the field. Throughout the present discussion there is an apparent inclination to push the analysis to the limit and, insofar as possible, to interpret the behavior of cellular elements in terms of chemical and physical forces at the molecular level. The author's point of view may perhaps best be indicated by quoting directly a few sentences from the preface. "Cytology" he says "began by describing what sort of things cells were. It continued by inferring what things happened inside them.

Its last and longest task is to discover why these things happen. . . . Finding out *why* things happen in cells is an entirely different matter from finding out *how* they happen. The one problem is a matter of skill and common sense. The other takes us into a new element." The new element, it would seem, is that in which chemists and physicists flourish. But even in this medium we shall indeed be fortunate if the *whys* of today do not become merely the *hows* of tomorrow. When they cease to do so perhaps science will have reached its goal. Be that as it may, the new orientation in which deductive reasoning is recognized as having a valid role, is heartily approved by J. B. S. Haldane, who points out in an appreciative foreword that "this attitude has long been normal in chemistry and physics," and adds that its introduction into biology is a sign of the growing unity of science. Indeed Haldane goes so far as to suggest that "it is perfectly possible that 'Recent Advances in Cytology' marks a turning point in the history of biology."

At the present stage of the analysis it would seem that any account of how cells divide must take cognizance of centrosomes and centromeres, remarkable entities which have much in common. The centrosome lies outside the nucleus; the centromeres, each of which is in a sense the very heart of a chromosome, lie within. Surrounded by media which are not isoelectric with them these bodies, in common with other cell organs, develop surface charges which are subject to fluctuations coincident with changes in the pH of cell fluids. Calculations of Haldane are cited to show that the resultant attractions and repulsions must be of a magnitude that could account for some of the observed phenomena of cell division. When the centrosome has divided and the daughter centrosomes have become oriented at the poles of the spindle—a liquid crystal which transmits electrical forces with maximum efficiency—the similarly charged centromeres are forced into a relatively neutral position in the equatorial plane. As the polar repulsions decline the centromeres divide and then, repelling each other, drag their respective half chromosomes to opposite poles. In this process we have first a centrosome spindle, then a centrosome-centromere spindle, and finally a centromere spindle. The apparent absence of observable centrosomes in the higher plants may mean that in them the centrosomes are small or that they do not respond to ordinary fixing and staining methods. Another possibility is that their function may be taken over by some other element in the cell. If the interpretations are sometimes lacking in complete and rigid proofs, they are at least based on a definite hypothesis with postulates than can be tested.

The general character of the explanation (or description) which Darlington offers for the main features of mitosis can be inferred from

the account of behavior of centrosome and centromeres. A few salient points may be mentioned for contrast with the familiar statements in conventional texts. When the chromosomal threads first appear at prophase they are already double. Their visibility at this stage is probably brought about through dehydration of chromatin that has been in a dispersed state. There is at no time a continuous 'spireme,' each chromosome (pair of chromatids) being independent throughout mitosis, as it probably is during resting stages. Shortening of chromosomes which occurs in late prophase is essentially due to a process of spiralization, relics of which can be detected in the partial uncoiling at telophase. Each chromosome has a centric constriction marking the site of the centromere or 'spindle fiber attachment,' and frequently secondary constrictions which indicate the position of 'nucleolar organizers.' At metaphase the centromere is always in or on the spindle but arms of longer chromosomes are free to wave to and fro in the current of streaming cytoplasm. Despite occasional appearances to the contrary, division of the chromosome, or better separation of chromatids, always begins at the centromere and progresses toward the distal ends of the arms as the daughter centromeres push apart. The apparent size of a chromosome, perhaps determined in some degree by the tightness of coiling, may be influenced by factors external to itself, as shown in properly selected hybrids.

It is in dealing with meiosis, which is the main theme of the book, that the extent of recent advances and the wealth of authenticated data are most impressively presented. Here, too, interpretive ingenuity is afforded ample scope. The treatment is critical, and to a degree historical, but scant notice is taken of alternative views or ideas that are thought to have been superseded. According to Darlington "the difference between meiosis and mitosis consists in the external mechanism which acts twice getting *out of step* with the internal mechanism which acts once. . . . The two forms of division are distinguished by a simple physiological difference."

In the prophase of meiosis the chromatin threads that appear are not double and are $2n$ in number. Polarized (bouquet stage) or not, the homologues unite in pairs, chromomere by chromomere, beginning usually, but not necessarily at the centromere. There may be terminal or intercalary segments in which union does not take place at all. This is particularly likely to be the case in long chromosomes where division into chromatids if initiated before synapsis is complete may inhibit further lateral fusion. In this connection the time element is an important one. During the early phases of meiosis homologous chromosomes are wound around each other—'relationally coiled'—and the pairs of chromatids into which each divides show a similar coiling,

but in the opposite direction. The origin of chromatids, however, results in a readjustment of internal attractions so that the two pairs tend to fall apart. But in doing so they produce an increased tension in the longitudinal direction which is frequently sufficient to snap one of the strands, the two ends of which can then partially uncoil. This creates a still greater stress in the other pair and one of them also breaks at the same place. The four free ends now make new unions by virtue of their terminal attractions and 'crossing-over' is thus effected. It follows that the two chromatids that may sometimes be seen to lie across each other in a chiasma are not the two that broke. After having been formed a chiasma may move along (like a noose) toward the end of an arm to become terminalized, but in doing so it may not cross the centromere. The formation of chiasmata and the processes involved in their terminalization are responsible for many pages that require close application on the part of the reader. It may be inferred that the Belling hypothesis is no longer tenable. Similarly the distinction of equational and reductional divisions in meiosis has been relegated to a position of merely historical interest, since the chromatids that separate following chiasmata formation represent a mosaic of materials that entered at synapsis and consequently some pairs of homologous genes are separated at the first division and others at the second.

It would be misleading to leave the impression that the volume is limited to a consideration of the mechanics of cell division and analysis of intercellular forces. There is an extended discussion of the nature and genetic implications of ring formations, and consideration of the origin and behavior of polysomics and polyploids, creation of new species, changes in chromosome complements, evolution of sex, hybrid vigor and sterility. Most of these subjects are treated with considerable adequacy, and if at times the treatment leaves a lingering doubt it never fails to be thought provoking. On the whole the book is rather heavily weighted on the side of plant cytology, but justly so for work has been most active in that field. It may be due to a subconscious botanical bias that table 12 relating to a number of plants and some fifty animals is labeled "Provisional Classification of Annuals and Plants."

The book concludes with four appendices: a provocative resumé entitled *Interpretation*; three pages of references on cytological technique; a glossary of many, but not all, of the special terms employed; and a bibliography of some 1700 titles.

C. H. DANFORTH
Stanford University

NEW BOOKS AND PUBLICATIONS

CONCEPTS AND PROBLEMS OF PSYCHOTHERAPY, by Leland E. Hinsie with a preface by Nolan D. C. Lewis. 1937. Size, $6\frac{1}{4} \times 9\frac{1}{4}$ inches. Pages xv + 199. Illustrated. Bibliography. Index. Cloth. Price, \$2.75. Columbia University Press, New York.

The purpose of this book is stated in the preface: "to indicate the general conceptions that prevail with respect to the structure and functions of the mind, and to show, as well as possible, what influences these conceptions have had upon the problems of psychotherapy." The introduction gives a general survey of psychotherapeutics, and comments on the extent and nature of psychotherapeutic problems. Succeeding chapters discuss the psychoanalysis of Freud, the psychobiological attitude of Meyer, the psychology of individuation by Jung and individual psychology by Adler. Dr. Carney Landis has contributed a chapter on statistical evaluation of psychotherapeutic methods.

FEEDING BEHAVIOR OF INFANTS. A Pediatric Approach to the Mental Hygiene of Early Life, by Arnold Gesell and Frances L. Ilg. 1937. Size, $7\frac{1}{4} \times 10\frac{1}{2}$ inches. Pages xii + 201. 200 illustrations. Bibliography. Index. Analytical index to illustrations. Cloth. Price, \$4.50. J. B. Lippincott Company, Philadelphia.

Feeding behavior may be regarded as the major key to the understanding of infant personality. Intake of food rather than its assimilation is the subject of this study. Its problems are psychological and have to do with adjustments which the infant as an individual must make prior to the time when the food reaches his stomach. By frequent systematic observations of a group of healthy babies, patterns of feeding behavior have been determined and a schedule of norms drawn up to serve as diagnostic criteria. These criteria are points of reference for detecting and defining individual characteristics. The subject matter of this volume is divided into three parts: the behavior aspects of nutrition, the growth of feeding behavior, and the regulation of feeding behavior. Wide variations from the norms are illustrated in the appendix which contains a number of biographic summaries showing the feeding history and behavior of different infants.

DAS CORPUS GENICULATUM EXTERNUM EINE ANATOMISCHE-KLINISCHE STUDIE, by Manuel Balado and Elisabeth Franke. 1937. Pages IV + 118. Size, $6\frac{1}{4} \times 10\frac{1}{4}$ inches. 123 illustrations, some of them in color. Index. Price, RM 36. Julius Springer, Berlin.

The corpus geniculatum externum is described as to its origin and differentiation, gross anatomy, histology, comparative anatomy, nerve tracts and blood supply.

MEDICAL RELIEF ADMINISTRATION. The Experience in Essex County, Ontario, by R. E. Holmes. 1937. Size, 6×9 inches. 55 pages. Essex County Medical Economic Research, Windsor, Ontario.

Those workers who are concerned with medical relief administration will be interested in this report describing policies and procedures with respect to the development of medical administrative techniques in Essex County.

THE LEAGUE OF NATIONS, INFORMATION SECTION, TWENTY-FIFTH SESSION OF THE HEALTH COMMITTEE. 1937. Size, 8×13 inches. 16 pages. Columbia University Press, New York.

The Health Committee held its twenty-fifth session from April 26th to May 1st, at which time it discussed and approved its next 3-year program. The new program is largely a continuation of the work already being done and is divided into two categories: permanent activities and those intended to deal with topical problems.

THE PRESPLenic FOLD

H. D. OBRIEN

Department of Anatomy, McGill University, Montreal

THREE FIGURES

The frequent occurrence of a more or less well-developed fold of peritoneum springing from the anterior surface of the gastro-splenic ligament, and the absence of any adequate description of it in the standard textbooks, led to an investigation of its incidence in a consecutive series of subjects in the dissecting room at McGill University.

This fold was first described by Reid ('12), who found it in 90% of fetuses examined. Subsequently, in 1913, he described the fold in an apparently normal adult, and mentioned that he had observed it in other adult subjects. He called the main fold the 'presplenic,' and described as 'subsplenic' a fold running from the presplenic to the diaphragm under the anterior pole of the spleen. This appears to be the only reference to the fold in man in the literature I have been able to consult. Straus ('36) in his survey of the viscera in primates makes no mention of the fold.

In the present investigation, thirty consecutive adult white bodies were examined with the results shown in table 1. It will be seen that the fold was present in some degree in twenty-nine out of thirty bodies. In only four of these could it be said to be a direct continuation of the great omentum. The presence of a cavity in the fold was only sought in fifteen bodies, and in these a cavity was more or less well developed in five and could not be demonstrated in ten. The factor of sex was not taken into consideration in estimating the incidence of fold or cavity.

In spite of the small number of bodies examined, it would seem that some degree of development of the fold is very common, if not actually constant.

In addition to the adult bodies, six fetuses were dissected, and the fold was found in the two oldest, each of which was about 120 mm. in C.R. length, while in one fetus of about 75 mm. there was a slight indication of the subsplenic part only. This is a lower incidence than that quoted by Reid, but the discrepancy may be due to dissection of younger fetuses than those used by Reid, who does not correlate the

TABLE 1
Presplenic fold. Incidence
Adult series of 30

	FOLD			
	Large	Small	Absent	Total
Cavity present	4	1	0	5
Cavity absent	5	5	0	10
Unspecified	11	3	1	15
Total	20	9	1	30

Fetus series of 6

Fold present in 2; absent in 4

Embryo series of 3

Fold absent in 3

incidence of the fold with the age of the fetuses. Apart from this discrepancy in incidence, the folds found in the fetus correspond closely to Reid's description (fig. 1).

In three serially sectioned embryos of 10 to 19 mm. no indication of the fold was found.

In the adult the fold is very variable in size and shape, and may be masked by deposits of fat, or by adhesions. It is not easily seen in routine dissections of the abdomen unless the left costal wall and attached diaphragm are cut away up to the level of the xyphi-sternal articulation before the stomach is touched.

In well-developed examples (fig. 2) the presplenic fold is a fan-shaped double layer of peritoneum from 2 to 5 inches



Fig. 1 Fetus, 120 mm. The liver has been removed, leaving the stomach (a) in situ. The presplenic fold (b) is seen lying over the spleen, and is prolonged downward as the omental process (c), and to the left under the spleen as the subsplenic fold (d).

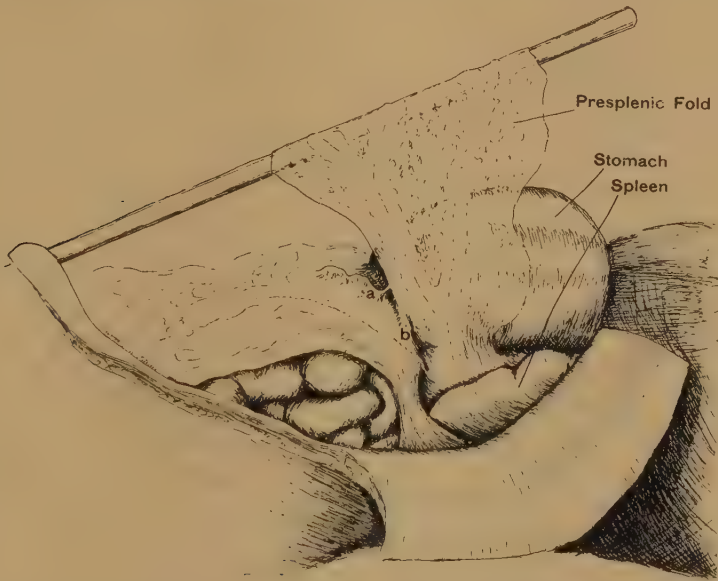


Fig. 2 Adult. The abdominal wall and liver have been removed. The stomach and spleen are pulled well forward. The presplenic fold has been thrown across a glass rod. The omental (a) and subsplenic (b) prolongations are poorly developed.

in depth, attached to the anterior surface of the gastro-splenic ligament along a line about $\frac{1}{2}$ inch from the greater curvature of the stomach, and extending from the upper to the lower end of the ligament. At its antero-inferior end the fold splits into two parts, of which the larger runs to the left as a sub-splenic fold under the anterior pole of the spleen, and blends with the phrenico-colic ligament, on which it may be traced in some cases as far as the diaphragm. The smaller fold is a more direct continuation of the presplenic, and rapidly loses itself on the anterior surface of the great omentum. These two subsidiary folds are less constant and much less well-developed than the presplenic fold itself.

The presplenic fold was generally draped over the antero-superior border of the spleen, to whose gastric or diaphragmatic surface it was not infrequently adherent. In other cases it was adherent to the anterior surface of the stomach, so that it appeared to spring from this surface rather than from the gastro-splenic ligament, to which, however, it could always be traced. Again in some cases the tip of the fold was adherent to the diaphragm, and thus enclosed the lower pole of the spleen in a sort of pocket.

The fold was generally supplied by two or more branches of the splenic or left gastro-epiploic artery.

In the fetuses showing presplenic folds no cavity in the fold was demonstrable, but in five out of fifteen adults a cavity was found (fig. 3). This cavity, when present, was a prolongation of the lesser sac, except in one case where there was a definite cavity in the fold, which had no communication with the rest of the peritoneal cavity, although there were no obvious signs of inflammatory adhesions. In the other cases the cavity occupied a variable proportion of the fold, and communicated with the lesser sac by a wide foramen bounded below by a falciform fold containing the left gastro-epiploic artery.

The presplenic fold seems to be developed from the left side of the dorsal mesogastrium above the course of the left gastro-epiploic artery. It appears some time after the great omen-

tum, and is at first formed by the left layer of peritoneum alone, the right layer subsequently growing out in some cases to form the cavity of the fold. The reason for its development is as obscure as that for the development of the great omentum, but it would seem natural that these two parts should be separate from the first, owing to the course of the left gastro-epiploic artery between them. Assuming that the presence of an artery between the layers of a peritoneal fold

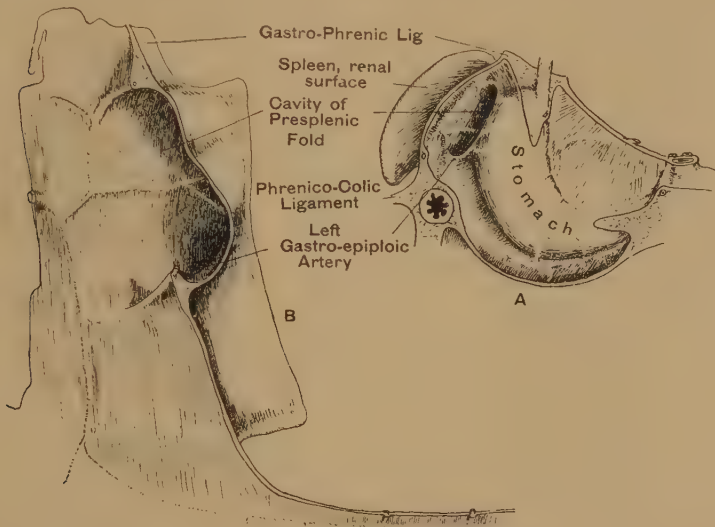


Fig. 3 A. Semi-diagrammatic view of posterior surfaces of lesser omentum, stomach, gastro-splenic ligament, and part of great omentum, to show foramen opening into cavity of presplenic fold. B. Posterior aspect of gastro-splenic ligament showing presplenic fold with cavity. Drawn from actual specimen.

can limit its growth, it follows that in certain cases where the vasa brevia running to the greater curvature of stomach are well developed or closely placed, then the presplenic process would naturally be partly or completely suppressed. The tendency for these arteries to suppress the growth of the fold would naturally be greater for the inner layer than for the outer, which would account for the lower incidence of the cavity in the fold than of the actual fold itself.

The subsplenic fold, generally well marked in the foetus, is often poorly developed in the adult, and generally appears to be merely a part of the phrenico-colic fold. Furthermore, the appearances in the fetus tend to bear out the supposition that the subsplenic fold is largely taken up in the development of the phrenico-colic fold, and is probably in part responsible for its development in the adult.

The small, omental process of the system is irregular, and of interest only inasmuch as it may contain a prolongation of the cavity of the presplenic fold, or may in some cases become continuous with the left free border of the great omentum.

Clinically the presplenic fold may be of importance in isolating left subphrenic effusions, and if adherent to the diaphragm or spleen, it might form a vascular obstruction to the surgeon attempting to remove the spleen.

For these reasons it would appear to be desirable to include some mention of the presplenic fold in the standard descriptions of the peritoneum in the gastro-splenic region.

SUMMARY

1. A system of presplenic, subsplenic and omental folds of the peritoneum in the gastro-splenic region is described, and its incidence in a series of specimens noted.

2. The presence of a cavity in the presplenic fold is described.

3. Some aspects of the development of the presplenic fold are considered, and the possible clinical significance mentioned.

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UTERINE CONTRACTIONS AND THE TRANSPORT OF SPERM IN THE RAT ¹

I. ROSSMAN

Department of Anatomy, The University of Chicago

TWO PLATES (EIGHT FIGURES)

INTRODUCTION

Antony van Leeuwenhoek described the independent motility of sperm in 1677, and some years later made the pioneer experimental study of their rate of ascent in the female reproductive tract. In a rabbit examined 15 minutes after the third completed coitus, he found sperm near the vaginal end, but not in the middle, nor apex of the uterus. In a similar experiment in which he delayed the examination for about 6 hours, the sperm were completely disseminated through the horn. This relatively slow ascent of sperm in the rabbit has since been confirmed by numerous investigators, although with the exception of Bischoff, no mention has been made of the great Dutch microscopist's observations.² His fundamental discovery that sperm could progress by the vibratile motion of their tails was made the basis for a generalized theory as to the

¹ Aided by a grant of the Rockefeller Foundation to The University of Chicago.

² Since Leeuwenhoek's communication appeared in a rare, early volume of the Philosophical Transactions of the British Royal Society, I quote a pertinent paragraph:

I bought a female Rabbet and let it take Buck 3 times in my presence (which was quickly done) and then killed it, but did not open the womb, till a quarter of an hour after; about an inch from the beginning of one of the Horns, I found a little fluid matter containing some few living animals of the Male seed, but in the parts where the Cornu joyns to the Vagina, the living Animals were very numerous; in the middle of the Cornu and toward the further end, there were no Animals at all, nor anything to be seen but a few blood globules swimming in a little fluid matter. The reason why the Animalis Seminis were not throughout the Cornu, I believe may be, that the Seed had not been long enough in the Womb, and therefore the animals had not time to disperse themselves all over it (p. 1128).

manner in which they traversed the uterus. This doctrine had a wide prevalence until recently, though several old investigations showed this could not be universally the case. One of these was Hausmann's (1840); he found semen throughout the uterus of a dog killed during copulation. Bischoff (1845) confirmed this in a bitch examined immediately after ejaculation, and later with Leuckart (1853) found sperm in the middle of the guinea pig tube $\frac{1}{4}$ hour after mating. The conclusion that the sperm had been helped along by uterine activity became inescapable, and Bischoff called attention to the contractions he had seen in such specimens.

Within the past decade there have appeared studies which again emphasize the rapidity of sperm transport in some animals. Hartman and Ball ('30) were able to aspirate sperm from the apex of the rat uterus within 100 seconds of a carefully determined ejaculation. Florey and Walton ('32) established fistulous connections to the uteri of a series of rodents. In the rat they observed "undiluted semen containing masses of spermatozoa" appearing at the fistulous opening 'immediately' after ejaculation; the same was true of the guinea pig. Evans ('33), using a similar technique on the dog, collected sperm from the fallopian end of the uterus in as short a period as 25 seconds after ejaculation. It must be conceded, therefore, that in some species the independent movement of the sperm is a negligible factor so far as their ascent of the uterus is concerned. An outstanding exception is the rabbit where the sperm take $1\frac{1}{2}$ to 2 hours to reach the distal end of the cornu (Parker, '31; Heape, '05).

We still lack, however, a picture of the behavior of the uterus at this time, and the manner in which the postulated contractions affect the semen within the organ. This gap in our knowledge of the events that occur between ejaculation and the appearance of sperm at the distal end of the uterus, has left room for various hypotheses. Most investigators have supposed that antiperistalses are the motive force (Bischoff, Leuckart, Evans, and others). Florey and Walton suggest that in the rat and guinea pig vaginal contractions are

mainly responsible. Hartman ('32) observes that in the distended rat uterus, at least, any type of contraction would be sufficient to churn up the contents. Since practically nothing is known of the uterine and vaginal contraction waves in most forms, the possibility is open that the mechanism differs in the various mammals. The present paper will report some observations on contracting uteri of rats shortly after coitus, both in vivo and in histological material.

MATERIAL AND METHODS

The animals were white and hooded rats, about 4 to 6 months of age. Oestrus was determined by examination of the vaginal outlet and smears. Suitable animals were then mated one or more times; some unmated specimens were also studied. After an ejaculation, the animals were removed from the males, and injected subcutaneously in the nape of the neck with a light dose of sodium amytal (8 mg. per 100 gm. of body weight). This method of anesthesia affects uterine contractions less than others I have tried; ether, notably, may completely abolish such activity. After receiving the injection they were returned to the mating cage, where they continued to copulate for 5 or more minutes, while the anesthesia was being induced. They sometimes received another ejaculation during this interval. When ataxia supervened, the animals were removed, and a uterine 'horn' exposed by suitable incision. The organ was examined under flowing, warmed Ringer's, using the quartz-rod method of transillumination devised by Knisely ('36).

To secure histologic specimens of these contracting uteri, I have utilized the method described by Daron ('36). Ninety-five per cent alcohol, chilled to about -60° with solid carbon dioxide, was poured over the viscus in situ. This brings the uterus to a rapid standstill at any desired point in the passage of a contraction wave. The icy-white organ, which may be partially or completely frozen through, is clipped out and thawed in a suitable fixative. It is then run up in the usual way. This procedure maintains the form relations of the con-

traction as it was observed in life through the binocular microscope.

OBSERVATIONS ON THE SEMEN-CONTAINING UTERUS

The uterus of the rat at heat contains a clear fluid which distends the organ, sometimes to an enormous degree. The extent of the dilatation varies from one animal to another, even though they be examined at comparable periods. With quartz-rod illumination, a very bright light can be passed through the uncontracted uterus, more than sufficient for microscopic observations. The uterine contents of unmated animals are quite transparent, but this is no longer the situation 10 minutes after an ejaculation and subsequently. Instead, the formerly homogeneous liquid is seen to contain numerous irregular particles buffeted about by the contractions. These vary in size and number in different specimens. After three or four matings, they may attain a diameter of almost $\frac{1}{2}$ cm., and become visible through the uterine wall to the unaided eye. Although it is difficult to follow any particular one throughout a contraction, they apparently maintain their integrity.

This heterogeneity of the uterine contents can be observed in our histological specimens (figs. 3 and 4). Here the particles are seen to be loose aggregates of wavy, irregular threads in which numerous sperm are embedded. Presumably we are dealing here with the same phenomenon that occurs in the formation of the vaginal plug, namely, the coagulation of seminal vesicle secretion by the action of the prostatic enzyme (Camus and Gley, 1896). Because of the dilution the semen undergoes in utero, the clots formed therein are of a much looser texture as befits the liquid environment. Probably the coagulum in its earliest phase is quite dispersed, and is later rendered more compact by the uterine contractions. As the illustrations show, their segregation from the rest of the mixture may become very pronounced.

The activity of the uterus varies from specimen to specimen, even with a standardized technique. One occasionally sees a

uterus in which wave succeeds wave, with almost no period of quiescence. As a rule, brief periods of rest lasting from 20 to 60 seconds, intervene between contractions; they are usually very rhythmic. Peristalses which commence in the tubal end of the uterus are the norm; other types of contraction are rare. Antiperistalses were observed in but one of a dozen animals, and occurred in conjunction with the usual contractions. They are certainly not characteristic of the rat uterus. A wave takes about 10 seconds to progress down the horn, though this may vary by a few seconds; in an individual specimen the time for the contraction phase remains fairly constant.

Two types of peristalses can be differentiated in the rat uterus. The more frequent one is illustrated in the first figure. The contracting area here involves about 1 cm. of the cornu; in life it appears as a constricted opaque band. In the other type, a smaller segment of uterus contracts, as is illustrated in figure 2. In the course of this wave, only 4 to 5 mm. of the muscle is contracting at a particular instant; in vivo it appears as a progressing node, and is perhaps associated with a greater degree of uterine dilatation. As with other variables, each type is characteristic of a particular specimen. When a large segment contracts, as in the type I wave, the uterus undergoes a slight torsion on its mesometrial axis; this becomes less pronounced the smaller the active area.

Although there are intergrades, it is necessary to distinguish the two types of contractions for reasons other than the morphological, since each has somewhat different effects on the uterine contents. When a wave commences, the segment involved shortens, constricts, and turns opaque due to optical changes in the muscle. The particles, which serve as an easily visible index to the direction and speed of the uterine liquor, are set in motion and proceed vaginalward. As the wave progresses, relaxation ensues at the fallopian end, and a certain amount of the uterine contents returns to its starting place, instead of continuing the journey down the uterus. This 'aspiration' particularly affects the smaller particles, and presumably the free sperm, which are possessed of less inertia.

The larger particles are usually driven to the opposite, cervical end. Since a smaller area is involved in the type II contraction, the tendency to return to the relaxing tubal end is more pronounced, and fewer particles make the complete journey. It should also be noted that the conditions for the piling up of seminal vesicle coagulum are more favorable in the type I wave.

In this latter contraction, most of the particles are transported to the vaginal end, which becomes markedly dilated. The time taken to make this portion of the trip is slightly less than that of a contraction wave, since the particles precede the driving segment. It accordingly varies with the specimen in hand, and may be as little as 4 seconds. The return toward the tube is more rapid, barely a second or two being necessary for the completion of the circuit. In a uterus 4 cm. in length, therefore, the particles may be hurtled along at a velocity greater than 2 cm. per second. The reason for this rapid return becomes obvious when the pressure relations within the uterus are analyzed. At the moment that the dilated vaginal end is commencing to contract down on the numerous particles there, we can roughly picture the uterus as being composed of three segments, in each of which the state of the uterine muscle differs. At the vaginal end, where the wave is ending, the pressure on the contents is greatest. The segment next above it is relaxing, and here the pressure is rapidly falling. At the fallopian end, which has been quiescent for a few seconds, a temporary equilibrium has been reached. It is under these conditions that the greatest pressure differential is set up within the contracting cornu, and the passive transport of semen is most rapid. The velocity thereby imparted to the sperm would be sufficient to carry them at least a short distance into the tube.

A more aimless churning up of the semen characterizes the type II contraction wave, where the current tubalward is set up earlier and is relatively stronger. Figure 2 illustrates a uterus in which such a contraction was halted when it had progressed about halfway down. At this time there existed

two well-marked currents proceeding in opposite directions from the narrowed active segment. This condition is maintained in the histological specimen, where the whorled pattern of the coagulates indicates the directions of the uterine liquor. Although the speed of these currents is not nearly so great as those set up by the more powerful band-like contractions, they are nevertheless sufficient to make the independent lashing about of the sperm insignificant. Since rapid, tubally-directed currents are set up by a contraction moving vaginalward, it is obvious that there is no necessity for invoking antiperistalses as the motive force in sperm transport.

PLASTIC CHANGES

The usual method of fixation does violence to the form relations that exist in the rat uterus at heat, since it fails to preserve the organ in the distended state and, in addition, sets up agonal contractions in the muscle. Unless a freezing technique is used, one may have to resort to such maneuvers as tying off a loop of the viscus preparatory to the use of the fixative. The method used by Daron in his study of the macaque uterus correctly preserves the conditions in the rat. A more rapid freezing can be obtained with isopentane chilled with liquid nitrogen, as recommended by Hoerr ('36), although the cold alcohol is adequate to halt contractions in the thin rat uterus. Most of the specimens illustrated have been obtained by this latter method. Since the plastic changes undergone by the hollow contractile viscera have been adequately described only for the gut (Heitzmann, 1868; and subsequent investigators), I shall take the opportunity to briefly describe some of them in the rat uterus.

The excellence of the histological fixation is by and large dependent on the extent of the initial freezing. Pouring several hundred cubic centimeters of the chilled alcohol over the organ is not necessarily sufficient to freeze it through, except in the thinner portions. The unfrozen part of the uterus is presumably only sub-cooled, and shows none of the usual ice artefacts in histological sections—such as reticulate or vacuolated cells. The picture is more uniform, and the vacuolation

reduced after isopentane, as compared with alcohol. The organ can be thawed in any suitable fixative, such as formol-Zenker, and the usual picture is secured in the unfrozen areas.

Figures 1 and 2 illustrate the changes that occur in contracting uteri, and the two types of waves already mentioned, as seen in longitudinal sections. Figure 2 demonstrates the nodal contraction. The constricted area is about 4 mm. in extent, and its structure is quite different from that seen in the relaxed portions. In the non-contracting sites, the wall is thin and stretched (fig. 6). Most of the elements of the mucous membrane tend to be oriented parallel to the lumen under the influence of the dilatation. This is especially true of the connective tissue framework, whose fibers and nuclei, except where they ensheath a gland, are thus disposed. The simple, widely-spaced glands run very obliquely through the mucosa, and frequently exhibit a sharp angular twist just before opening to the lumen. The veins and arterioles similarly pursue an oblique course, and the slight tendency of the arterioles to spiral is accentuated. The lumen is large, and approximately circular; in different specimens the lining epithelium varies from columnar to cuboidal, with the degree of distention. When the epithelium is cuboidal, its nuclei may be pushed over to a position at right angles to the usual axis. Both muscle coats are thin, and the serosa is closely applied to the longitudinal tunic. Between the two layers of muscle is a stratum vasculare, in which the larger blood vessels run. In a distended area these arteries and veins may be flattened, and appear oval in transection; unlike their branches to the mucosa, they show less spiralling.

In the contracting area, both muscle coats are considerably thickened (fig. 8). The mucous membrane is heaped up to a height of 1 mm., and its elements are considerably reoriented. The stroma is denser, and its cells and fibers are arranged perpendicularly to the lumen, except in a narrow sub-epithelial zone. Glands and blood vessels are approximated and straightened; the slight spiral of the arterioles has disappeared. The lumen is decidedly smaller, and irregular in shape. It is lined by a typical tall columnar epithelium. The

serosa over the contracting muscle is thickened and wrinkled. The veins in the stratum vasculare are pinched and irregular in bore; associated with this is a dilatation of the endometrial veins. A more detailed investigation of the changes in the arteries is being pursued.

Figure 1, which illustrates the type I contraction in a less dilated uterus, exhibits similar plastic changes. The active area is about 1 cm. in extent, and the mucous membrane is thrown into numerous folds. Its other features are similar to those of the previous specimen, except that the longitudinal muscle runs quite obliquely, a condition which produced the torsion observed in the living organ. It is of interest from another standpoint, since it came from an animal that had been mated once, and yet the uterus contains no semen. There are, instead, numerous blood cells, especially erythrocytes; this condition has been noted by Long and Evans ('22). We can only conjecture what failing in the mechanism was responsible for this; it serves, at any rate, as a control on the observations made with semen-containing uteri. The distribution of the reds is precisely what would be expected from our knowledge of the currents in such uteri. Most of them are concentrated at the dilated end, and relatively few are found caught in mucosal folds elsewhere. In vivo, the pink uterine liquor was propelled back and forth in the manner already described.

The following table will summarize the plastic changes illustrated in figures 1 and 2.

	FIGURE 1		FIGURE 2	
	Contracted	Relaxed	Contracted	Relaxed
Height of mucous membrane	0.35-0.60 mm.	0.12-0.14 mm.	0.8-1.0 mm.	0.17-0.25 mm.
Height of lining epithelium	26 μ	17 μ	26 μ	20 μ
Thickness circular muscle	180 μ	50 μ	290 μ	90 μ
Thickness longitudinal muscle	85 μ	45 μ	110 μ	36 μ
Thickness of serosa	28 μ	10 μ	7-20 μ	7 μ

These are average measurements from celloidin material.

DISCUSSION

The observations were made 10 minutes after mating and subsequently, and though there is no reason to suppose that the uterus behaves in an essentially different way at the time of copulation, such a possibility cannot be excluded. We may reasonably disregard antiperistalses in a consideration of the transport of sperm in the rat since they are so uncommon. I have not seen in either unmated animals or in those examined shortly after coitus, any evidence of leakage from the uterus, such as would occur if the cervix were open. This situation cannot, of course, obtain at the time of mating, but we can only guess as to the role of the cervix at that time. It may be that it plays no more active a part than to be temporarily open. It should be noted that it is unnecessary to postulate cervical or vaginal activity as a mechanism involved in the transport, and until such is shown to be the case, we must rely on the behavior of the uterus for explanation.

A normal uterine peristalsis would be sufficient to rapidly 'aspirate' sperm from the vagina when the cervix opens. We would then be dealing with a tubular system that temporarily extended into the vagina, instead of ending at the cervix, as under the conditions of our observations. At one phase in the progress of the contraction wave, pressure differentials exist that move semen to the tubal end of the uterus in a second or two. With the cervix open, we can suppose that first a spurt of uterine liquor mixes with the vaginal semen, and then 'aspiration' occurs. This would suffice to account for the swift en masse ingress of sperm that is known to occur.

It should be pointed out that no great accumulation of fluid is necessary for the rapid transport of semen. The observations of Florey and Walton (op. cit.) on rats with apical fistulae would indicate this. Although these authors make no mention of the extent of fluid accumulation in such uteri, it could not have been marked. After a stab is made into the oestrous rat uterus, small jets of fluid are ejected during the contraction, except when the area about the puncture is immediately involved. Such uteri lose most of their dilatation

after a time; nevertheless, in Florey and Walton's specimens, the same rapid transport of a less diluted semen occurs. The great distention of the rat uterus is then not a necessarily significant phenomenon for the transport of sperm, and such uteri may be compared with those of other species where it does not occur to the same extent (compare Evans, loc. cit.).

The emigration of leukocytes that occurs in the uterine wall at oestrus may be significantly correlated with the clots of semen we have observed in our specimens. Although these are loose-textured structures, infiltrated with the uterine liquor, they nevertheless maintain the semblance of integrity during the contractions. It seems probable, therefore, that sperm associated with these structures, and embedded in their meshes, are not available for fertilization. These inutile elements may be autolyzed by the large number of leukocytes furnished at this time, as has been described for the guinea pig by Stockard and Papanicolaou ('19).

I should like to thank Prof. G. W. Bartelmez for the aid and suggestions he has generously extended to me, and Dr. M. H. Knisley in whose laboratory much of this work was performed.

SUMMARY

1. Contractions and their effects in semen-containing uteri of rats are described as observed *in vivo*.
2. These contraction waves can be fixed for histological study, by the use of the freezing technique devised by Daron ('36).
3. The plastic changes undergone by such uteri are described.
4. The seminal vesicle component of the semen is coagulated in the uterus. The clots and excess sperm found therein may be the *raison d'être* of the leukocyte emigration (compare Stockard and Papanicolaou, '19).

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PLATES

PLATE 1

EXPLANATION OF FIGURES

I am indebted to Mr. R. D. Bensley for the photographs.

1 Longitudinal section of a rat uterus showing the type I contraction. Blood cells are for the most part limited to the dilated end. $\times 7.8$.

2 The type II contraction in longitudinal section. There were two well-defined currents moving the semen in opposite directions at the moment of freezing. $\times 7.8$.

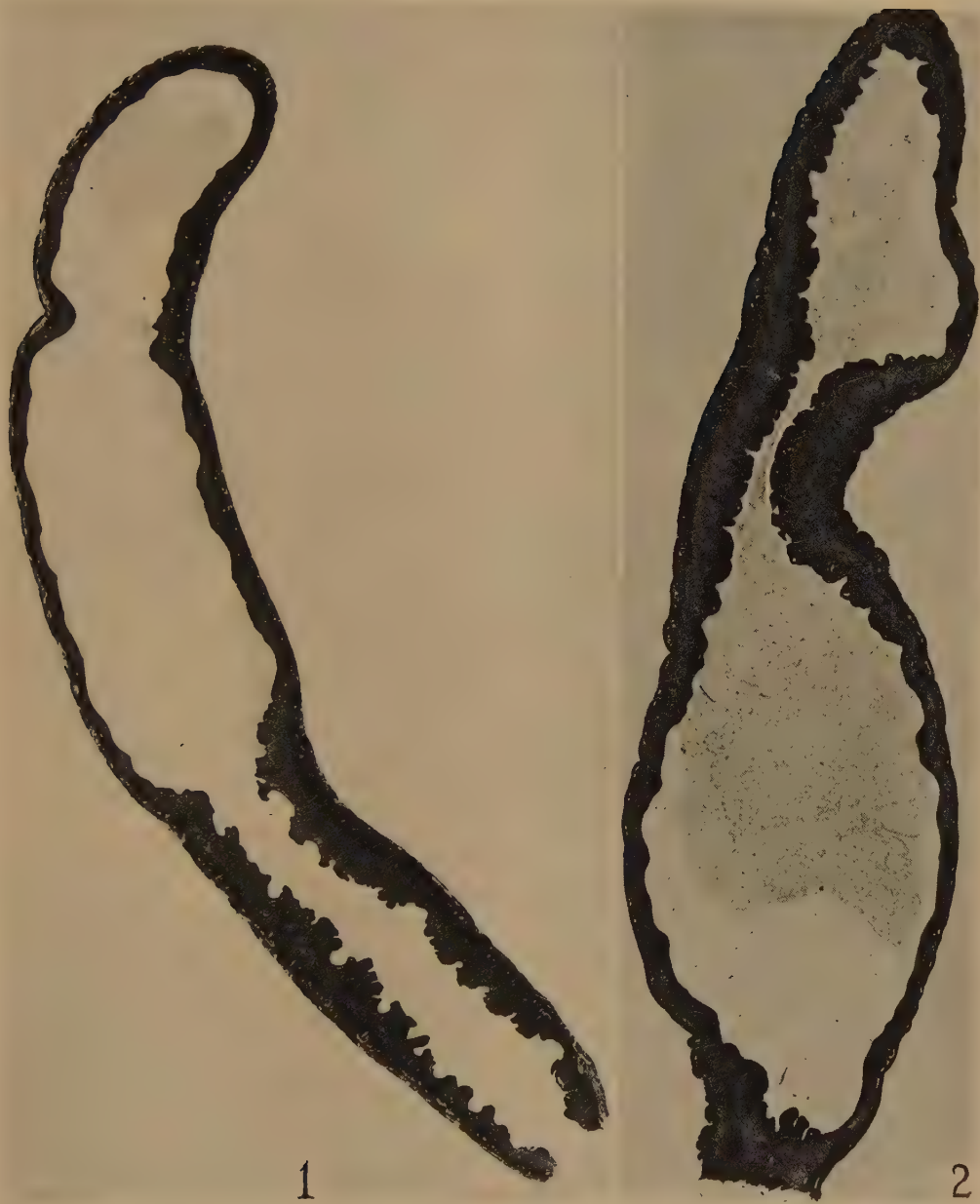


PLATE 2

EXPLANATION OF FIGURES

3 Cross section of relaxed oestrous uterus. The wall is thin and stretched; the large lumen contains a 'clot' of semen. $\times 15$.

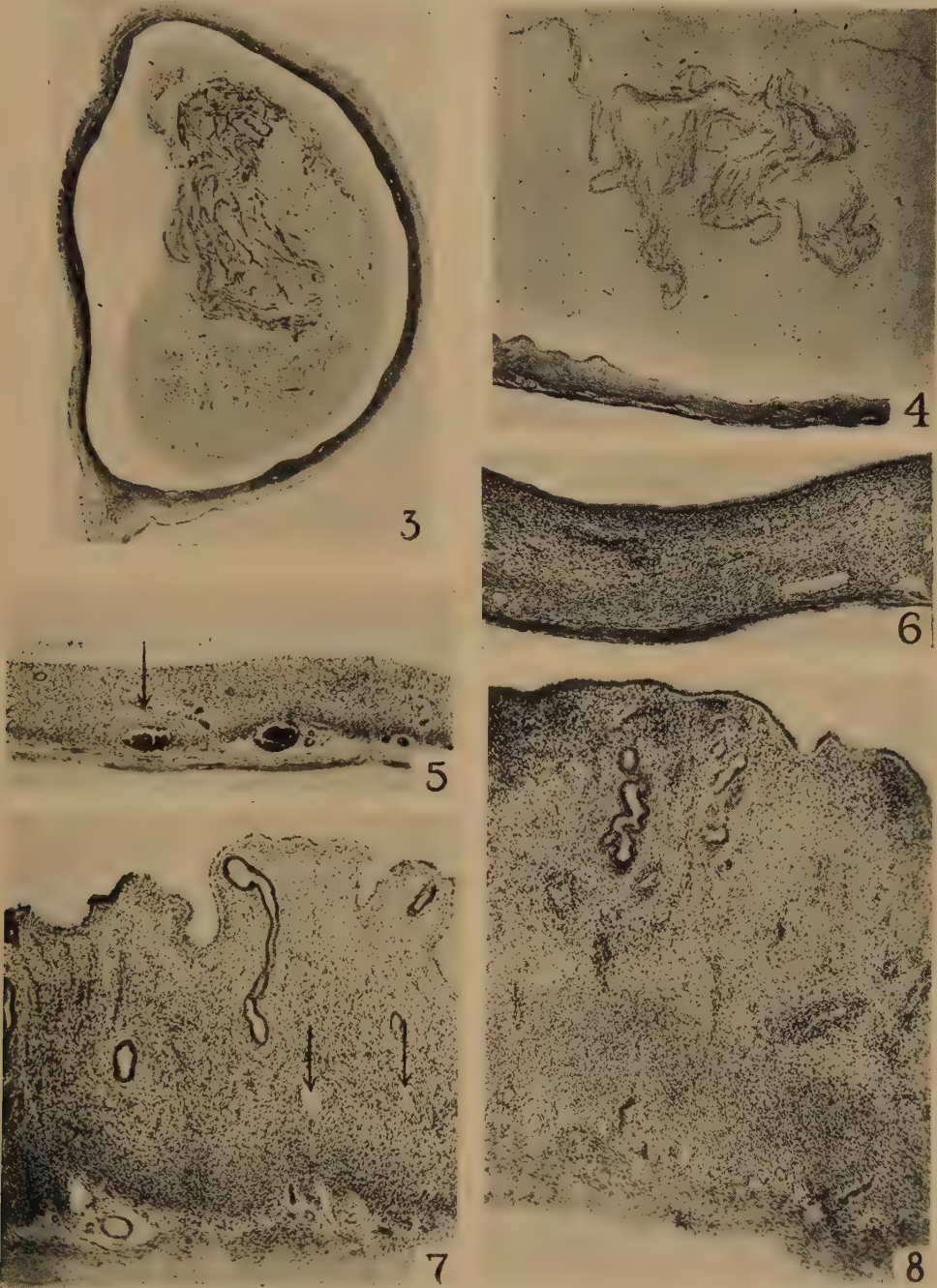
4 From the dilated end of a contracting uterus, cut in longitudinal section. The loose mesh of seminal vesicle coagulum measures over $2\frac{1}{2}$ mm. in diameter. From a specimen frozen in isopentane, thawed in formol-Zenker. $\times 15$.

5 The dilated portion of the uterus illustrated in figure 1. The veins are cut in cross section, but appear flattened. Between them an arteriole ($\downarrow$) spirals obliquely through a stroma oriented parallel to the lumen. $\times 50$.

6 From the relaxed area of the uterus illustrated in figure 2, showing a similar stretching. $\times 50$.

7 From the contracted area of the specimen shown in figures 1 and 5. The endometrium is tall and wavy, and the stromal elements are oriented perpendicularly. The veins ($\downarrow$) are dilated at the point of juncture of mucous membrane and muscle. $\times 50$.

8 The uterine wall of the specimen illustrated in figures 2 and 6, in an area of contraction. The plastic changes are similar to those of the preceding specimen except for the mucosal folds which are less pronounced. $\times 50$.



A CASE OF LATERAL HERMAPHRODITISM IN MUS MUSCULUS

ELIZABETH FEKETE

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

ONE FIGURE

There have been two cases of hermaphroditism in mice reported in the literature. Danforth in 1927 described a gynandromorph mouse with a normal ovary and uterine tube on the left side and a testis, ductus deferens and seminal vesicle on the right side.

Blotevogel in 1932 observed an externally normal female mouse in which the right gonad was an ovary and the left a testis.

A third case was noted in a 2-month-old mouse of the Little-Murray dilute brown strain.

The external genital organs of this mouse were that of a normal female, and a vaginal plug was noted 2 days before the animal was killed. Under the dissecting microscope the organs of the right side appeared perfectly normal. Unfortunately no microscopic preparation could be made from the ovary and oviduct because these organs were cut up and were transplanted into a male mouse for experimental purposes. Only later was the abnormality of the left side noted. The testis was small and was situated somewhat low in the abdominal cavity. Figure 1 shows the position of the organs, and makes further gross description unnecessary.

Microscopical sections showed that the right uterine horn was normal and well developed. The testis showed very active spermatogenesis; spermatogonia and spermatocytes were

present, but there were no spermatozoa. The caput epididymis and vas deferens were normal. The rudimentary left uterine horn was attached to the vas deferens. The seminal vesicle showed hypoplasia and was appended to the cervix. The vagina was normal.

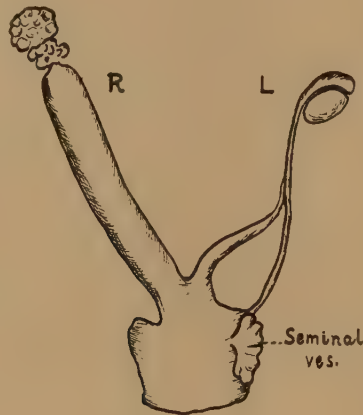


Figure 1

It is interesting to note that all three of these animals were normal females externally and all were lateral hermaphrodites having an ovary on one side and a testis on the other side.

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THE STAINING OF PARAFFIN SECTIONS OF NERVOUS TISSUES WITH ACTIVATED PROTARGOL. THE ROLE OF FIXATIVES ¹

DAVID BODIAN

Department of Anatomy, The University of Chicago

THREE FIGURES

The degree of selectivity of silver impregnation methods depends to a large extent upon qualities conferred upon the tissues by preliminary treatment, especially the initial fixation. This fact is very often overlooked, although it has long been known. It has recently been demonstrated to be true of osmium as well as of silver methods in the important paper of Owens and Bensley ('29). Modification of the impregnation technique itself, as well as of the initial fixation, have both often been employed to obtain differential staining of various nervous structures. However, in all the block impregnation methods and in most of the section impregnation methods, it is extremely difficult to control completely the course of the impregnation so as to obtain constant results.

Using a silver impregnation method for paraffin sections described elsewhere (Bodian, '36), it has been found that apparently much of the selectivity of the stain can be made to reside in the initial fixation. It has thus been possible to stain differentially many diverse nervous structures, without any modification, and with apparently complete control, of the impregnation method. This has been found to be a much more satisfactory method than attempting, by modifying the impregnation technique, to stain structures which have not been differentially fixed. An attempt was then made to determine which fixing agents are best suited for the selective

¹ This research was aided by a grant to The University of Chicago from the Rockefeller Foundation.

staining of various nervous elements, and of divers parts of the central nervous system of various animal groups.

The fixatives which have been found most useful, and their particular applications, are presented in the hope that they will be of value to investigators of the nervous system. Many of the results were obtained by the method of trial and error, especially in the early stages of this study. It was not possible to try everything and many interesting failures are not reported. It is hoped that sufficient data are presented to indicate the possibilities inherent in the method, and perhaps to suggest lines of further experimentation, which may be stimulated by the needs of special problems. The fixing agents generally used for reduced silver methods are unfortunately usually inadequate for refined cytological analyses, but at present they are the only reagents which can be used in fixing tissues for the differential staining of nervous structures. In a subsequent report, the effect of the mode of preservation upon the stainability of such sensitive indicators of adequate preservation as the vestibular club endings and boutons terminaux in the goldfish will be described in detail.

THE IMPREGNATION METHOD

The impregnation method as originally described has been used essentially without modification up to the present time. However, the use of a large variety of fixatives has brought out the fact that intensity of staining depends in part on the fixing agent used. Thus, it has been found that with some material the gold-toning is not always as essential as formerly believed. On the other hand, it has been found possible to accentuate certain structures which stain very lightly, by means of double impregnation, as described below. It is also possible, after the protargol-copper bath, to treat the sections with ammonical silver as in Rogers' ('31) modification of the Bielschowsky method. The staining by this method, however, is not as satisfactory, in general, as with the hydroquinone reduction.

The impregnation technique is summarized as follows:

1. Remove paraffin with xylol and run sections through absolute alcohol and 95% alcohol to distilled water.

2. Place sections in a solution of 1% protargol (silver albumose) containing about 4 to 6 gm. of metallic copper (copper wire, for example) per 100 cc. of solution; 12 to 48 hours at 37°C. Wash in distilled water. *The protargol-copper bath can be used only once.*

3. Place in reducing solution:

1 gm. hydroquinone

5 gm. sodium sulphite (or 5 cc. formol)

100 cc. aq. dest.

5 to 10 minutes. Wash thoroughly in at least three changes of distilled water. If toning with gold is not desired, the sections are now dehydrated and mounted in balsam. For double impregnation, the sections, after having been washed, are placed in a second bath of fresh protargol and fresh copper and then treated as in 2 and following sections.

4. If greater contrast is desired, sections may be toned in gold, as follows:

a. After washing, following the hydroquinone bath, the sections are placed in a solution of 1% gold chloride, containing 3 drops of glacial acetic acid per 100 cc. of solution, until sections are decolorized (usually 2 to 5 minutes). Wash in aq. dest.

b. Place sections in 1% or 2% oxalic acid, until entire section has a faint purple or blue tinge (from 2 to 5 minutes usually). Wash in aq. dest.

c. Remove residual silver salts in a solution of 5% sodium thiosulphate (5 to 10 minutes).

5. Wash thoroughly in aq. dest., dehydrate, and mount in balsam.

For some material, especially the peripheral nervous structures, counterstaining with a 1% aqueous solution of acridine red for a few seconds after step 4 has been found advantageous. The differentiation is carried out in the final dehydration with 95% and absolute alcohol, and is rapid. Other counterstains may also be used of course, such as Mallory's connective tissue stain or Mallory-azan. Mounted sections of old formalin material may be treated with 5% acetic acid overnight before staining with activated protargol. This tends to prevent impregnation of collagenic fibers.

Celloidin sections as well as paraffin sections can be used, but it is advisable to remove the celloidin before staining.

Whole mounts of unembedded tissue cultures (Weiss and Wang, '36) and of mesenteries have also been stained successfully.

Fixing agents

1. Formol	10 cc.
Normal salt solution	90 cc.
2. Formol	5 cc.
Glacial acetic acid	5 cc.
80% ethyl alcohol	90 cc.
3. Formol	5 cc.
Trichloroacetic acid	1 to 2 gm.
80% ethyl alcohol	90 cc.
4. Formol	5 cc.
Formic acid	2 to 5 cc.
80% ethyl alcohol	90 cc.
5. Formol	5 cc.
Strong ammonium hydroxide (sp.gr. 0.90)	1 cc.
80% ethyl alcohol	90 cc.
6. Absolute alcohol (ethyl)	95 cc.
Glacial acetic acid	5 cc.
7. Absolute alcohol (ethyl)	100 cc.
Trichloroacetic acid	1 to 2 gm.
8. Methyl alcohol (absolute)	95 cc.
Glacial acetic acid	5 cc.
9. d'Ancona's fluid.	
95% alcohol	40 cc.
Pyridine	20 cc.
Chloral hydrate	2.5 gm.
Aq. dest.	40 cc.
10. Huber's fluid	
95% alcohol	100 cc.
Trichloroacetic acid	1.5 gm.
Mercuric chloride	3 gm.
11. Rectified bichromate mixtures (modified from Foley, Hunt and Sackett, '36)	
5% potassium dichromate, Regaud's fluid, or Zenker-formol	80 cc.
Pyridine	10 cc.
Methyl alcohol	10 cc.
Strong ammonium hydroxide	1 cc.
12. Carnoy's fluid.	
13. Bouin's fluid.	

The results of a great number of trials have shown that the most generally satisfactory fixative is formol-acetic-alcohol (formula 2). The attempt has been made in the selection of fixatives for the staining of various nervous elements, to find a fixative which produces the least amount of artifact while permitting selective and sharp staining with protargol. Since fixatives containing chrome salts generally prevent staining with reduced silver, recourse must be had to other mixtures which penetrate well and produce as little shrinkage and distortion as possible. It is possible to use potassium dichromate mixtures recognized to be of cytological excellence, and still to obtain selective staining with protargol, but this can be done only by adding rectifying substances (Foley, Hunt and Sackett, '36), which perhaps may impair the best fixing qualities of the original reagent (see formula 11).

Whenever possible the fixatives are perfused through the blood vessels as previously described (Bodian, '36).

MAMMALIAN TISSUES

Embryos. Excellent results from the point of view of general fixation and sharpness of differentiation of nervous elements have been obtained with pig embryos of all stages from 10 mm. on, with formulae 2, 3 and 4. Formula 2 gives perhaps the best results for both central nervous system and peripheral nerve, while formula 4 appears to give finer detail in the central nervous system.

Adult tissues. Cerebral cortex. Formulae 2, 6 and 9. Pyramidal cells appear to be best stained after fixation with formula 2, nerve fibers after formula 9.

Cerebellum. Formulae 2, 6, 7, 8, 9. Climbing fibers and baskets are best stained after fixation with 6 and 7, basket axons with 9, and Purkinje cells and mossy endings with 8. It has appeared remarkable that the use of methyl alcohol and acetic acid (formula 8) should give an extremely rich impregnation of Purkinje cell dendrites approaching the pictures obtained with the Golgi method, whereas ethyl alcohol and acetic acid (formula 6) gives a much less complete impregnation of the finer ramifications of the Purkinje

cell dendrites, and a much better staining of climbing fibers. It is possible that this difference may be due to impurities in the alcohols, as well as to different fixing properties of ethyl and methyl alcohol, respectively.

Brain-stem. Formulae 1, 2 and 3, preferably perfused according to the technique described previously (Bodian, '36). Perfusion with 80% alcohol, as formerly employed, is satisfactory only if the tissues are subsequently extracted in frequent changes of 95% alcohol for several weeks. Fixation with the above formulae, however, has been found superior even to this method.

Spinal cord. Formulae 1, 2, 3, 7. Cells, fibers and neurofibrils are best shown with formulae 2, 3 and 7, of which formula 7 appears to give the most intense staining. The best preparations of endfeet of Held in mammalian material have been obtained in the spinal cord of the cat by perfusing 1 liter of 10% chloral hydrate and subsequently placing pieces of cord in 95% alcohol for 2 to 3 weeks (changing frequently), as described previously (Bodian, '36). It is quite probable that after perfusion with chloral hydrate, formulae 1 and 11 may also give good staining of endfeet, since direct immersion fixation with these fixatives, without preceding perfusion with chloral hydrate, gives occasional staining of endfeet.

Spinal ganglia. Formulae 2, 3, 7 and 11.

Coeliac ganglion. Formulae 2, 3, 10 and 12.

Peripheral nerve. Formulae 2, 3, 10 and 11. Good results have also been obtained with formulae 1 and 7.

Muscle endings. Excellent preparations of motor endplates have been obtained in striated muscle after fixation by perfusion with formula 1. A minimal quantity of normal saline (75 cc. for an adult cat) should precede the formol-saline in the perfusion. Perfusion with formula 2 also gives good results. Counterstaining with acridine red after the silver impregnation, as described above, is useful with this material. The stapedius muscle of the cat has been used principally in this work (figs. 2 and 3).

Other peripheral nerve endings. Vestibular nerve endings in the cristae and maculae of the goldfish have been brilliantly stained after fixation with formula 3 (fig. 1). This mixture is also recommended for mammalian auditory and vestibular end-organs and for endings in teeth.

Intergemmal endings and intragemmal endings in the taste buds of the macaque have been well stained by the method of double impregnation, following fixation by perfusion with formula 1.

Suprarenal gland. Nerve fibers and their endings can be well stained in the suprarenal medulla following fixation with formulae 7 and 10. Fixation of the parenchyma is excellent with formula 7 and with formula 2, after which nerve fibers can also be well stained. However, nerve endings are more difficult to obtain with formula 2 than with formulae 7 and 10.

Hypophysis. Formulae 1 and 2. Excellent staining of nerve fibers in the pars intermedia and pars nervosa of the hypophysis of the macaque and of the cat has been obtained after perfusion with formulae 1 and 2. The finest fibers and nerve endings in the pars intermedia are best demonstrated by double impregnation following fixation with formula 1.

Retina. The few experiments so far made have produced selective staining only of the horizontal cells in the retina of the cat after fixation by perfusion with formula 1.

THE NERVOUS SYSTEM OF FISHES

For this work, *Carassius auratus* (goldfish), and *Ameiurus melas* (catfish) have been used.

The best general staining is obtained with formulae 2 and 3. Good staining of nerve cells and fibers is also obtainable after formulae 1 and 13. Endfeet of Held are best stained after fixation with formulae 1 and 13, but can also be stained after fixation with other reagents. Alkaline fixatives are worthless for the nervous system of fishes as also for most other nervous tissues.

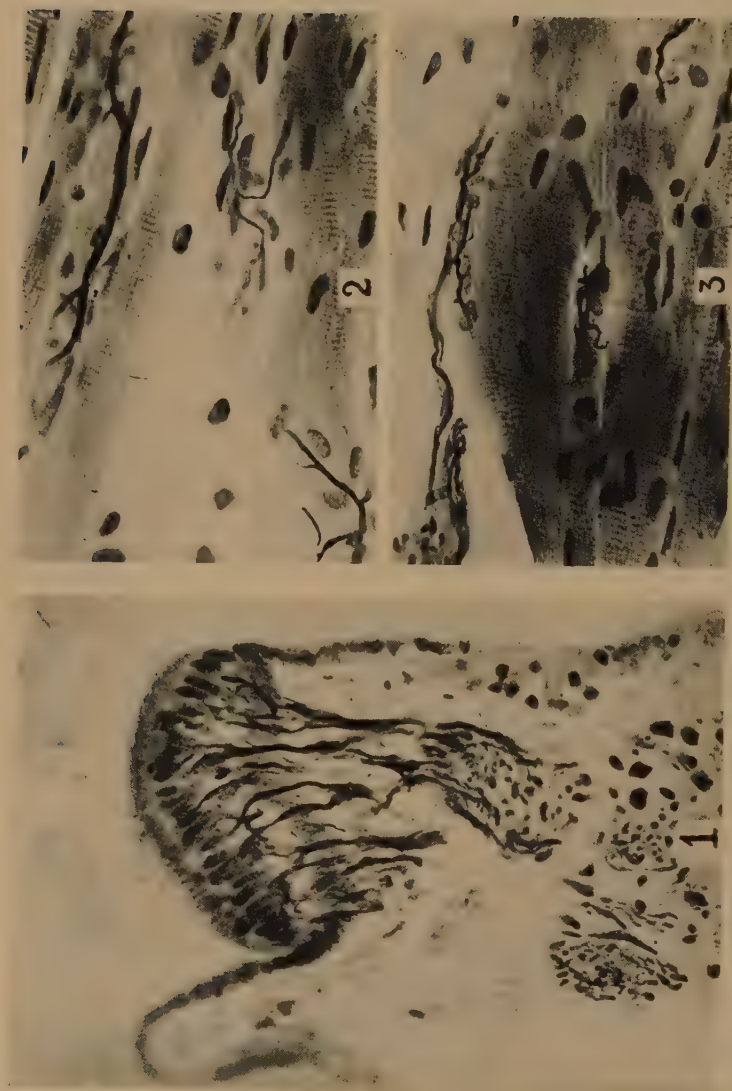


Fig. 1 Nerve endings in epithelium of crista ampullaris of the goldfish. Fifteen micra section, stained with activated protargol after fixation in formol (5%)-alcohol (80%)-trichloroacetic acid (1%). $\times 480$.

Fig. 2 and 3 Nerve endings in the stapedius muscle of the cat. Fifteen micra section, stained with activated protargol after fixation by perfusion with 10% formol-saline. $\times 480$.

THE NERVOUS SYSTEM OF AMPHIBIA

Adult *Rana pipiens* and adult and larval *Amblystoma tigrinum* have been used for this study. The best general fixative is formula 4. Formulae 5 and 9 are also excellent for the central nervous system. The finest fibers in the gray matter of the central nervous system are stained after fixation with formula 1, which is also satisfactory for peripheral nerves.

THE NERVOUS SYSTEM OF REPTILES

The best general results are obtained with formulae 2 and 3, but formulae 1 and 13, and other acid fixatives can also be used successfully. These findings have been obtained with the rattlesnake (*Crotalus atrox*).

THE NERVOUS SYSTEM OF INVERTEBRATES

Sharp staining of the nervous system of the crayfish, which was used in these experiments, was difficult to obtain. The best results were obtained with acid fixatives such as formulae 2, 3, 12 and 13. Double impregnation is helpful, as is also reduction with ammoniacal silver, as in Rogers' ('31) method, following the protargol bath.

The author's acknowledgment and sincere thanks are tendered to Mr. Robert D. Bensley for the preparation of the photomicrographs, to Dr. A. A. Pearson for assistance in the experiments with pig embryos, and to Mr. David B. Clark for technical assistance.

SUMMARY

By the use of proper fixatives preceding impregnation with activated protargol, it is possible to stain selectively many diverse elements of nervous tissues. It has been found that by using a single impregnation technique, practically all of the selectivity of the stain can be made to reside in the initial fixation, with extremely constant results. The most useful differential fixatives are presented for the staining of 1)

mammalian embryos, 2) adult mammalian central and peripheral nervous systems, 3) nerve endings in the central nervous system, in striated muscle, in the hypophysis, in the suprarenal medulla in taste buds, and in the vestibular sense organs, 4) central nervous system of invertebrates, fishes, amphibians and reptiles.

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UNFERTILIZED HUMAN TUBAL OVA¹

GREGORY PINCUS AND BARBARA SAUNDERS

Biological Laboratories, Harvard University

ONE PLATE (TWO FIGURES)

During the past year we have, through the courtesy of Drs. F. A. Pemberton and G. V. Smith of the Brookline Free Hospital for Women, obtained two postoperative specimens of human fallopian tubes which yielded ova upon flushing with a balanced salt solution.

On March 17, 1936 we examined the excised uterus, right fallopian tube, and right ovary of a 40-year-old white patient whose last menstruation occurred on February 28, 1936. This patient had had regular 28-day cycles up to the time of the operation complicated only by excessive bleeding in the few cycles preceding the operation. The excessive bleeding appeared to be due to uterine fibroids. The uterine endometrium was in the early secretory stage.

The tube was flushed with Pannett-Compton solution and in the washings the ovum of figure 1 was recovered. The egg was mounted in serum upon a slide with raised coverslip, a still photograph taken (fig. 1 a), and then photographed once every 8 seconds for 1 hour and 45 minutes with a 16-mm. cinematographic camera at 37.5°C. The ovum was then fixed in Bouin's solution, but unfortunately was lost early in the process of dehydration. Upon fixation the egg became considerably shrunken and we are inclined to believe that its loss was due to its final complete collapse.

This ovum bears a remarkable resemblance to an egg recovered also on the eighteenth day of the cycle by Lewis ('31).

¹ This investigation was aided by a grant from the Josiah Macy, Jr. Foundation.

It will be seen from the figures that the egg contains a dark coarse cytoplasm with several large dark yolk particles. The vitellus is in close contact with the zona as in the Lewis ovum and the zona is slightly irregular but shows the striations due presumably to the radial position of the coronal processes. This egg is somewhat smaller than the Lewis ovum, measuring 125 and 115 μ in its longest and shortest diameters compared with 151 and 145 μ given for the Lewis egg. The corresponding vitellus diameters are 110 and 95 μ compared with a mean of about 109 μ for the tubal ova described by Allen, Pratt, Newell and Bland ('30) and 133 and 138 for the Lewis ovum.

The cinematographic strip revealed definite cytoplasmic movements in the egg. These were in the nature of the somewhat localized 'bubbling' movements seen in degenerating ova rather than the regular cyclosis observed in normal living eggs in other mammals (Pincus, '36). Further evidence of atretic processes within the ovum was the fact that the cytoplasm retained its dark coarse appearance after incubation (fig. 1 b). In recently ovulated unfertilized rabbit ova, for example, the yolk masses do not remain clumped after culturing but disperse throughout the cytoplasm giving the vitellus a smoothly granular appearance. Two- to 3-day-old tubal ova of the rabbit, on the other hand, usually do not exhibit marked yolk dispersion. The slightly damaged portion of the zona at one end of the ovum (fig. 1 a), indicates that destruction had probably begun though no phagocytes were observed inside the zona.

The ova of most mammals enter the uterus from the third to fourth day after ovulation (Hartman, '32; Pincus, '36). Since this ovum was obviously long post-ovulatory but tubal it seems likely that the ovulation occurred in this case between the fourteenth and sixteenth days of the cycle or between 12 and 14 days before the end of the cycle.

The second ovum (fig. 2) was obtained on March 6, 1937 from the left fallopian tube of a white patient aged 30 whose last menstrual period occurred on February 15, 1937. The

cycle immediately previous had lasted for 26 days, and her cycle lengths ordinarily varied between 26 and 30 days. The ovaries and tubes of this specimen were normal with a recent corpus luteum in the left ovary. The uterine endometrium was in the late proliferating stage and showed some dysplasia.

The tube was washed 3 hours after excision and the egg was found in the washings surrounded by a mass of granulosa-like cells. No true corona was present, however (fig. 2), and it is our opinion that the corona had been lost and that the egg became adventitiously entangled in the surrounding cell mass. We believe that this cell mass represents the remains of the cumulus oophorus shed at ovulation and still retained in the tubes. The egg, mounted on a slide, in Tyrode's solution was photographed fresh (fig. 2 a), placed in a small drop of egg albumen, and fixed with Bouin's solution. In the fresh specimen the practically spherical ovum measured $121\ \mu$ in diameter, the vitellus $111\ \mu$. Practically no shrinkage of the zona occurred in the fixed specimen since the zona extended through twelve sections cut at $10\ \mu$. The vitellus of the fixed specimen measured $100\ \mu$ in the maximum diameter.

We believe this ovum to have been more recently shed than the preceding one. In the first place it contained no perceptible dark yolk masses in the fresh state (fig. 2 a). Second, the zona was not injured, nor did it show in the fresh state the radial striations so clearly; in older unfertilized rabbit ova, for example, the radial markings are more obvious than in recently shed ova (compare Pincus, '30, fig. '9). Third, the polar body is still present in this ovum (fig. 2 c), whereas we saw no sign of a polar body in the preceding ovum, e.g., it had presumably degenerated. The presence of a late proliferating endometrium would indicate furthermore that corpus luteum function had scarcely begun whereas the early secretory endometrium of the preceding case indicates an early active corpus.

At the same time we believe this egg to be at least 1 day old and more probably 2 days. The nuclear spindle (fig. 2 b) shows definite signs of breakdown and an irregular polar

migration of certain of the chromosomes has occurred. Furthermore, in one section of the ovum we find a definite cytoplasmic extrusion (fig. 2 d) which is not a polar body since it is free of chromatin. Such extrusions are observed in degenerating tubal ova of the mouse at about 2 days after copulation (Charlton, '17) and Pincus ('30) observed similar extrusions in a set of rabbit ova taken at 40 hours after copulation in a rabbit, but never in earlier ova. The smoothly granular appearance of the egg cytoplasm (figs. 2 b and 2 c) indicates that marked degeneration had not taken place, however.

Accordingly we believe this second ovum to be at most 2 days old, which would place ovulation at the seventeenth day of this cycle. This would indicate ovulation at between the ninth and thirteenth day before the end of the cycle in this particular patient. Both of these ova, therefore, add to the evidence that ovulation in women, ordinarily occurs at about the middle of the menstrual cycle (Hartman, '36).

SUMMARY

Two unfertilized human tubal ova are described, one obtained on the eighteenth day and the second on the nineteenth day of the menstrual cycle. Judging from the condition of the ova and of the uterine endometrium ovulation is presumed to have occurred some time between 12 and 14 days before the end of the cycle in the first case, and some time between 9 and 13 days before the end of the cycle in the second.

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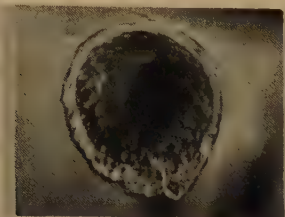
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PLATE 1

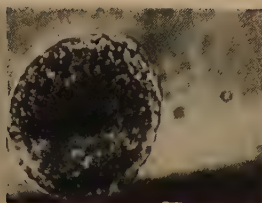
EXPLANATION OF FIGURES

1 Two views of the first unfertilized tubal ovum. $\times 200$. A. Still photograph of ovum made shortly after recovery from the tubes. Note slightly damaged portion at lower right. B. Enlargement from a cinematographic record. The clear, striated outer band is the zona pellucida.

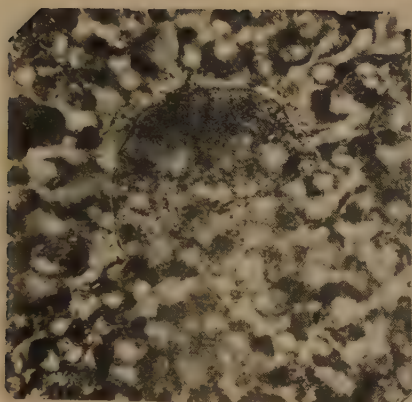
2 The second unfertilized ovum. A. The fresh ovum surrounded by granulosa tissue. B. Section through the fixed ovum showing the marginal chromosomes of the egg spindle. C. Section showing the polar body. D. Section showing the cytoplasmic fragment.



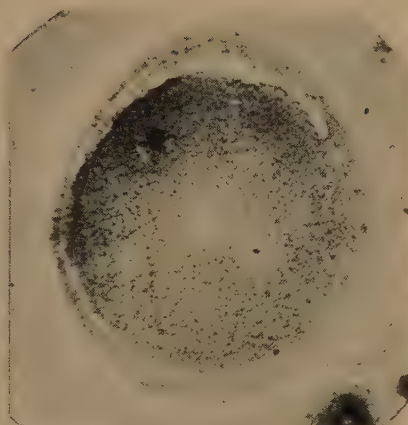
1A



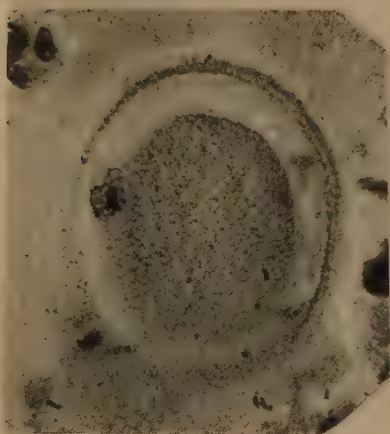
1B



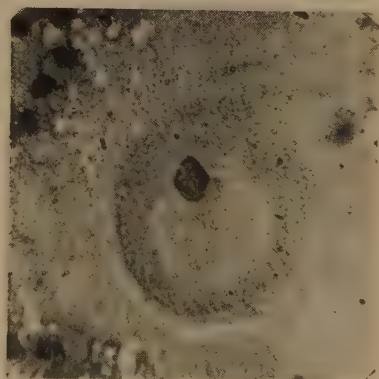
2A



2B



2C



2D

QUANTITATIVE EFFECTS OF THEELIN ON BODY GROWTH AND ENDOCRINE GLANDS IN YOUNG ALBINO RATS

CLAY B. FREUDENBERGER AND FRED W. CLAUSEN
Department of Anatomy, University of Utah, Salt Lake City

Because the results reported in the literature varied in some respects and were incomplete in others, the present study was carried out, dealing with the effects of estrogenic substances on body growth and the weights of the endocrine glands. In this experiment the animals were of known strain and age. A relatively large number of animals was used and biometrical methods were employed in evaluating the results.

MATERIALS AND METHODS

Two groups of immature female Wistar albino rats were injected with 200 I.U. (60 R.U.) of theelin in oil¹ daily, beginning at the twenty-first day of age. One group of twenty-five was injected for a period of 10 days and autopsied. Twenty-five litter-mate controls received an equal amount of olive oil. The other group of twelve rats was injected for a period of 5 days and sacrificed. Eleven controls received an equal quantity of olive oil.

According to D'Amour and Gustavson ('36), estrogenic substances dissolved in oil are more stable than aqueous solutions of these substances.

The biometrical interpretation used in the 10-day group is represented as the significance ratio in the table. A value of 3 or more is regarded as being significant, while one less than

¹ The theelin used in this experiment was kindly furnished by Parke, Davis & Company through the courtesy of Dr. Oliver Kamm.

3 is regarded as being of doubtful significance. In the 5-day group Fisher's short sample method was employed because of the smaller number of animals. A value of *P* of 0.05 or less is regarded as being significant.

*Effects of theelin injections on body growth and weights of endocrine glands.
(Injections of 200 I.U. daily for 5- and 10-day periods)*

ORGAN	DAYS	MEAN VALUES		DIFFER- ENCE	PER CENT CHANGE	SIGNIFI- CANCE RATIO	t	P
		Theelin	Olive oil					
Hypophysis, grams	5	0.00305	0.00240	+0.00065	27.08	10.6	4.113	0.01
	10	0.00440	0.00320	+0.0012	37.32			
Thyroid, grams	5	0.00667	0.00610	+0.00057	9.34	6.5	1.783	0.1
	10	0.00940	0.00790	+0.0015	19.03			
Thymus, grams	5	0.2372	0.2836	-0.0464	16.35	13.1	2.631	0.03
	10	0.2715	0.4324	-0.1609	37.21			
Suprarenals, grams	5	0.01315	0.01290	+0.00025	1.93	2.4	0.459	0.7
	10	0.01616	0.01699	-0.00083	4.89			
Ovaries, grams	5	0.01922	0.01688	+0.00234	13.86	2.0	2.145	0.056
	10	0.02202	0.02078	+0.00124	5.96			
Body weight, grams	5	46.6	46.0	+0.6	1.30	6.6	0.384	0.7
	10	61.7	68.4	-6.7	9.82			
Head weight, grams	5	6.8	6.65	+0.15	2.25		0.906	0.4
	10							
Body length, centimeters	5	12.03	12.01	+0.02	0.16	1.9	0.116	0.9
	10	13.40	13.56	-0.16	1.17			
Tail length, centimeters	5	9.47	9.5	-0.03	0.31	2.9	0.145	0.9
	10	10.49	10.92	-0.43	3.91			

OBSERVATIONS

Hypophysis. The average weight of the hypophysis of the 5-day group was 0.00305 gm. for the tests and 0.0024 gm. for the controls. The difference was a 27.08% increase in the test animals. This change was definitely significant, the value for *P* being 0.01.

The average weight of the hypophysis of the 10-day group was 0.0044 gm. for the tests and 0.0032 gm. for the controls. The test animals showed an increase of 37.32% over the controls. The significance ratio for the difference was 10.6.

These results were in agreement with those of other workers such as Leonard, Meyer and Hisaw ('31), Wolfe ('35), Wolfe and Chadwick ('36), Ellison and Burch ('36), Cramer and Horning ('36), McEwen, Selye and Collip ('36), and Halpern and D'Amour ('36). However, Leonard, Meyer and Hisaw did not regard the increase in weight found by them to be significant.

Thyroid. The change in thyroid weight at 5 days was not great. The tests had an average weight of 0.00667 gm. while the controls had an average weight of 0.0061 gm. The difference of 9.34% was probably not significant since the value of P was 0.1.

The thyroid gland of the tests of the 10-day group showed an increase in weight of 19.03% over the controls. The average weight of the thyroid gland of the test animals was 0.0094 gm. while that of the controls was 0.0079 gm. The change was definitely significant, the significance ratio being 6.5.

These findings substantiate those of Pincus and Werthessen ('33). Leonard, Meyer and Hisaw ('31) and Ellison and Burch ('36) found no changes.

Thymus. The thymus gland showed a 16.35% decrease in weight in the test group of the 5-day injection experiment. The gland of the tests had an average weight of 0.2372 gm. while the average weight of the gland of the controls was 0.2836 gm. The value of P was 0.03 so the difference was regarded as significant.

The thymus of the 10-day group showed more marked changes. The average weight of the glands of the test rats was 0.2715 gm. while that of the controls was 0.4324 gm. The difference was a 37.21% decrease with a significance ratio of 13.1.

Leonard, Meyer and Hisaw ('31) and Spencer, D'Amour and Gustavson ('32) found no thymic changes as a result of theelin injections. On the other hand, Golding and Ramierez ('28) found changes in agreement with the present work.

Suprarenal glands. The suprarenals of the 5-day group had an average weight of 0.01315 gm. for the tests and a weight of

0.01290 gm. for the controls. The 1.93% increase was probably not significant since the value for P was 0.7.

No definite weight changes in the suprarenals were found in the 10-day group. The tests had an average weight of 0.01616 gm. while the controls had an average weight of 0.01699 gm. The 4.89% decrease was of doubtful significance since the significance ratio was only 2.4.

Such workers as Leonard, Meyer and Hisaw ('31) and Spencer, D'Amour and Gustavson ('32) found no adrenal changes. On the other hand, Ellison and Burch ('36) used very large doses of estrogenic substances and found enlargement of the suprarenals.

Ovaries. The rats that were injected for 5 days with theelin had ovaries with an average weight of 0.01922 gm. while the controls had ovaries with an average weight of 0.01688 gm. The 13.86% increase in the tests was probably significant since the value for P was 0.056.

In the 10-day injection group the ovaries of the test rats had an average weight of 0.02202 gm. while the ovaries of the controls had an average weight of 0.02078 gm. The difference was an increase of 5.96% over the controls, which was probably not significant since the significance ratio was 2.0.

The general opinion is that injection of estrogenic substances for relatively short periods of time produces an enlargement of the ovaries. This was shown by the work of Wolfe ('35), Selye, Collip and Thomson ('35), Lane ('35), Ellison and Burch ('36) and Mazer, Israel and Alpers ('36).

Body weight. At autopsy the average body weight of the tests of the 5-day group was 46.6 gm. as compared to 46.0 gm. for the controls. The 1.3% increase was not significant since the value of P was 0.7.

The autopsy weight of the 10-day group was 61.68 gm. for the tests and 68.4 gm. for the controls. The change was a 9.82% decrease in the test animals. The significance ratio for the difference was 6.6.

These results were in accord with those of Spencer, D'Amour and Gustavson ('32).

Head weight. The tests had an average head weight of 6.8 gm. in the 5-day group as compared to 6.65 gm. for the controls. The 2.25% change was not significant, the value for P being 0.4. The head was not weighed in the 10-day group.

Body length. In the 5-day test group the average nose-anus length was 12.03 cm. while the 5-day controls had an average length of 12.01 cm. The 0.16% change was not significant. The value for P was 0.9.

In the 10-day group the nose-anus length showed no definite changes. The average for the tests was 13.4 cm. as compared to 13.56 cm. for the controls. The difference was a 1.2% decrease in the test animals. The significance ratio was only 1.9.

Tail length. The tail length of the 5-day group showed no changes. The tests had an average length of 9.47 cm. while the controls had an average length of 9.5 cm. The difference was only a 0.31% decrease in the test animals. The value for P was 0.9.

The tail length of the 10-day test group was smaller than that of the control group. The average for the tests was 10.49 cm. as compared to 10.92 cm. for the controls. The difference was a 3.91% decrease in the test animals. The change was probably significant since the significance ratio was 2.9.

DISCUSSION

There is convincing evidence to show that the changes in the endocrine glands as a result of theelin injection are due to stimulation from the hypophysis (Engle, '31; Selye et al., '35; Lane, '35; Halpern and D'Amour, '36; Nelson, '36). This would account for the enlargement of the thyroid and the ovaries (the hypophysis itself is enlarged). The lack of changes in the adrenals seems to indicate failure of stimulation from the hypophysis. Larger doses of estrogenic substances, on the other hand, have been shown to cause stimulation of the adrenals with resultant enlargement as demonstrated by Ellison and Burch ('36).

It is to be noted that body weight was decreased. It appears that either the growth-promoting hormone was inhibited, or

some other factor was operating to prevent its activity. An interesting observation is the fact that the changes in body growth reported by Freudenberger and Clausen ('37) after prolonged periods of injection have begun to become apparent at such an early date.

The initial effect of theelin injection appears to be stimulation of the hypophysis with liberation of increased amounts of thyreotropic, gonadotropic and adrenotropic (large doses of theelin) hormones. Later the hypophyseal hormones are decreased in amount due perhaps to the original over-activity of the gland. This then results in a smaller animal as shown by Freudenberger and Clausen ('37). However, there appears to be no initial increase in the amount of growth-stimulating hormone, or if there is an increase in amount of this hormone, its effects are neutralized in some manner.

We wish to call attention to the fact that in the present work the thymus gland became smaller in test animals and did so before the body weight decreased. The possibility of the thymus playing an heretofore unrecognized role in the chain of events presents itself.

SUMMARY

Two groups of immature female Wistar albino rats were injected with 200 I.U. (60 R.U.) of theelin in oil daily, beginning at the twenty-first day of age. One group was injected for a period of 5 days and autopsied, the other group for a period of 10 days and then sacrificed. Both groups had litter-mate controls which were injected with equal amounts of olive oil.

The hypophysis was significantly enlarged in the 5- and 10-day groups. The thyroid gland was enlarged in both groups, but significantly so in the 10-day group only. The ovaries were also enlarged in both groups, but significantly so in the 5-day group only.

The thymus gland was significantly decreased in weight in the 5- and 10-day groups. The body weight was significantly decreased after 10 days, the change being first significant after

6 days. The tail length was significantly smaller after 10 days of injection.

The head weight, suprarenal glands, and body length showed no significant changes in either group.

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THE EFFECTS OF VARIOUS GONADOTROPIC SUB-
STANCES AND THYROXINE ON THE OVARIES
OF HORNED LIZARDS (PHRYNOSOMA
CORNUTUM)¹

C. H. MELLISH AND ROLAND K. MEYER

Department of Zoölogy, University of Wisconsin

INTRODUCTION

Relatively few reports have been made concerning the physiology of the sexual cycle and the action of mammalian gonadotropic agents in female reptiles. Forbes ('34) obtained an hypertrophy of the ovaries and oviducts in immature alligators following administration of an unfractionated extract of the anterior pituitary gland of sheep. Similar results were obtained by Evans ('35) in the sexually inactive lizard, *Anolis carolinensis*, with this type of preparation and with a human pregnancy urine extract.

In another communication Mellish ('36) reported the data concerned with an attempt to determine the environmental factors influencing sexual periodicity in the horned lizard and to compare the effects of an anterior pituitary extract in this form with those in mammals. It was found that when females were kept under continuous exposure to electric light and at a temperature of 35°C. from the middle of January to the first part of March, there was no apparent stimulation of the genital system over that of controls which were kept in hibernation at a temperature of 5°C. However, when animals kept under conditions of high temperature and exposure to light, were injected with a total of 3 gm. equivalent of an

¹ This investigation was aided in part by a grant from the Wisconsin Alumni Research Foundation; grant administered by Roland K. Meyer.

unfractionated anterior pituitary extract (hog), the ovaries were markedly increased in weight, the oviducts were more thickened and convoluted and the glands of the mucosa were hypertrophied and seemed to be in an active secretory state.

In view of these findings it became of interest to determine the effect on the lizard ovary of fractions of anterior pituitary extracts known to have either a gonad-stimulating or inhibiting action in mammals. Extracts of the former type have been separated into the so-called follicle-stimulating and luteinizing fractions (FSH and LH) each of which has a characteristic action on the ovaries of the immature rat. The FSH fraction increases the weight of the ovaries and stimulates the growth of follicles whereas the LH fraction is without action when injected alone but when given with the follicle-stimulating fraction will cause luteinization and will augment the FSH (Fevold and Hisaw, '34). On the other hand Evans and his co-workers ('36) have reported the preparation of a pituitary fraction (antagonist) which would inhibit the gonadotropic action of pregnant mare's serum and certain anterior pituitary extracts. Since the results obtained by these workers led them to conclude that the antagonist specifically inhibited the FSH factor contained in injected preparations or elaborated from the animal's own pituitary, a preparation of the antagonist was injected with an LH extract to determine whether it would prevent the action of any FSH from either of these sources.

Since some of the preparations mentioned above are known to contain factors which in mammals act on glands other than the gonads, it was thought advisable to treat lizards with the serum of pregnant mares. This was used because it has been shown to be capable of increasing the ovarian weight and of causing luteinization and ovulation in the immature rat (Cole, '36). Furthermore, pregnant mare's serum has been demonstrated to have no effect on the thyroid (Greep, '35) and the presence of other gland-stimulating substances has not been reported.

Inasmuch as Eggert ('35) has found a close association of the thyroid and gonad cycles in lizards, it seemed advisable that attempts be made to determine the importance of the metabolic rate in effecting gonadal stimulation in the horned lizard. Accordingly, some animals which were hibernating at a low temperature were injected with an unfractionated anterior pituitary extract while others which were kept at a higher temperature were treated with thyroxine.

PROCEDURE

All animals were obtained from the vicinity of Dallas, Texas, in the middle of December and were kept under the following conditions until used in an experiment: The illumination (sunlight) was dim and diffuse during the day. There was no artificial lighting at night and the temperature varied from 5°C. to 18°C. Although the lizards were constantly supplied with mealworms, it is doubtful whether any food was taken since they remained in a relatively inactive and lethargic state.

The experiments were performed at various times from the middle of December to the middle of February. During this period horned lizards are normally inactive and their gonads in a state of retrogression.

Certain of the animals were injected with preparations of FSH, LH, pregnant mare's serum or thyroxine. Others were treated with preparations of both antagonist and LH fractions. All of these animals were maintained under the following conditions: On the first day of the experiment they were placed in a compartment in which the temperature was kept at 32°C. by a continuously lighted 16-candle power electric bulb. They were given an over-supply of mealworms and were watered every other day. Other lizards were injected with an unfractionated pituitary extract while hibernating at a temperature of 5°C.

The control animals consisted of three groups which were killed at different times. Two of the groups were subjected to high temperature and artificial light for 9 days. The third

group was kept until the time of autopsy under the same conditions as the animals prior to treatment. Since the degree of ovarian development and body weights of these groups were very similar, the data for each group have not been recorded separately.

The FSH preparation and LH preparations *a* and *b* were made from desiccated whole sheep pituitary glands by the method described by Fevold and Hisaw ('34). Extracts prepared by this method should be free of the thyrotropic fraction (Greep, '35).

The LH *c* and antagonist fractions were prepared from fresh hog pituitaries according to the method used by Evans and his co-workers ('36). They state that such an LH fraction contains small amounts of antagonist, thyrotropic, adrenotropic, growth and lactogenic factors whereas the antagonist is free of the lactogenic activity but does have traces of these other substances.

The unfractionated anterior pituitary extract which was used was a water extract of dried whole sheep glands.

The pregnant mare's serum was not extracted and was preserved in the frozen state.

The gonad-stimulating preparations were assayed on immature female rats and were found, with one exception (LH *b*), to evidence their characteristic action. This latter extract, while having no effect by itself, proved to be very weak in augmenting and luteinizing properties when injected with an FSH fraction.

The antagonist was shown to have inhibiting action when tested by giving intraperitoneal injections with pregnant mare's serum into immature female rats.

The thyroxine employed was a synthetic crystalline product manufactured by Hoffmann La Roche. It was dissolved in a 5% solution of aqueous alcohol for injection. A total of 2 mg. was chosen as the amount to be injected since it has been shown that the tissue metabolism of alligators was raised 150% following administration of 4.4 mg. of thyroxine (Hopping, '31) and because Gaddum ('29) demonstrated that in

rats 2 mg. of thyroxine would increase the oxygen consumption as much as 25%.

All injections in the lizards were made intraperitoneally once a day for 8 days and the animals were killed on the ninth day of the experiment. Body and gonadal weights were taken, the genital systems examined, the digestive tracts opened and contents examined, and the size of the fat bodies noted. The ovaries, oviducts and thyroids were preserved in Bouin's solution and later sectioned and stained with iron haematoxylin and eosin. The thyroids of animals used in this and other experiments are being studied and will be considered in another report.

RESULTS

The digestive tracts of all animals kept at a high temperature contained recognizable food material. In those kept at the lower temperatures any substance that was present could not definitely be identified as recently ingested food. All the lizards in both the experimental and control groups had fat bodies at the time of autopsy. The size varied with the individual.

The ovary of the horned lizard is much like that of the bird. There is little stroma and the major portion is composed of eggs in various stages of development. During the winter the ovaries of animals which have not been artificially stimulated (controls) are less than 1 cm. in diameter at their widest point. The eggs as seen by macroscopic examination vary from 0.5 to 2 mm. in diameter and are white and translucent. The oviducts of such sexually inactive animals are relatively straight and narrow.

The data concerned with both experimental and control animals are included in table 1. Since the body weights showed wide variation between groups, probably the figures concerned with the gram ovary/gram body factor are more representative of the effect of the treatment than are the actual weights of the ovaries.

Reference to table 1 will show that animals which were injected with the FSH fraction, LH preparations (a) and (c) with the combination of preparations LH (b) and antagonist ² and with pregnant mare's serum had ovaries which weighed several times more than those of the controls. One animal in the group injected with the LH (b) and antagonist preparations had ovulated as two degenerating eggs were found in the left oviduct. This is the only instance of ovulation which occurred. The general appearance of the ovaries of

TABLE 1

The effect of certain gonadotropic extracts and thyroxine on the ovaries of the horned lizard

TYPE OF EXTRACT	TOTAL AMOUNT	AVERAGE BODY WEIGHT	AVERAGE OVARY WEIGHT	GRAM OVARY/GRAM BODY
		<i>gm.</i>	<i>mg.</i>	<i>average</i>
FSH	2.0 gm. eq.	18	875	0.049 (8) ¹
LH a	1.5 gm. eq.	27	1606	0.059 (7)
LH b	1.5 gm. eq.	16	239	0.015 (4)
LH c	1.5 gm. eq.	12	781	0.065 (5)
INHIB.	1.0 gm. eq.			
LH b	1.5 gm. eq.	16	1381	0.086 (5)
PMS	1.0 cc.	21	1361	0.065 (7)
Unfract. AP ²	2.0 gm. eq.	20	118	0.0059 (5)
Thyroxine	2.0 mg.	15	37	0.0025 (5)
		18	103	0.0055 (15)

¹ Indicates the number of animals on which these averages are based.

² This experiment was conducted on animals hibernating at a temperature of 5°C.

all these groups was the same. About 50% of the eggs had a diameter of 3 to 7 mm. and were of a bright yellow color. The rest of the eggs varied from 0.5 to 2 mm. in size and were white and translucent. The majority of the weight increase is attributed to the extensive yolk deposition. The oviducts of these animals were more vascularized, more convoluted and wider than those of controls.

² Since entering these data some animals were received from the southern part of Texas. Part of these animals were killed and were found to contain partially stimulated ovaries. Others were injected for 4 days with the antagonist fraction (total 1 gm. eq. fresh gland). On autopsy the ovaries showed a very marked increase in weight and yolk content. This would indicate that the antagonist fraction alone is capable of producing the stimulation cited above.

Although the ovarian weights of lizards injected with preparation LH (b) were twice as great as those of controls, they were obviously much less than those of any of the other stimulated animals. None of these ovaries contained more than four eggs which exhibited yolk deposition and increased size. It should be noted that this preparation had been shown to be very weak in its luteinizing and augmenting properties when assayed on the immature female rat.

In those animals which were treated with thyroxine both the ovarian weights and the gram ovary/gram body factor were less than half as much as those of control animals. The eggs were less than 0.5 mm. in diameter; many were cloudy and opaque and appeared much like degenerating or atrophic material. Little, if any, increased activity was observed in these lizards. At the time of autopsy they appeared to be strong and healthy.

The lizards which were injected with an unfractionated pituitary extract at a temperature of 5°C. hibernated throughout the 9 days of the experiment. When brought into a warm room for autopsy, they began to move about within a few minutes and seemed to be in good condition. The weight and appearance of the ovaries were approximately the same as those of control animals. Apparently at least a part of the injected material had not been absorbed since the body cavities contained fluid which looked like the extract.

DISCUSSION

It has been shown in the experiments reported in this paper that treatment with FSH and LH fractions and with pregnant mare's serum produced ovarian weights that were much greater than those of non-injected animals. The weight increase is primarily to be accounted for by extensive yolk deposition.

In the case of the pituitary extracts there may have been small quantities of various other tropic factors present. Since such large amounts of material were used, it is conceivable that the stimulation might have been caused by some of these

agents rather than by the gonadotropic entities. However, since results of the same kind were obtained by the use of pregnant mare's serum, it seems probable that the gonad-stimulation in the lizard which was obtained by anterior pituitary gonadotropic fractions was not caused by other tropic materials contained in such extracts.

The small ovarian weights obtained in the lizard with luteinizing fraction LH (b) were directly correlated with the inability of this preparation to accomplish augmentation and luteinization in the immature female rat comparable to those produced by the other LH extracts.

The process of extensive yolk deposition in lizards following the treatments cited above is very similar to the effects of injecting an unfractionated gonadotropic pituitary extract into the dove and of giving FSH to the mature fowl (Riddle and Bates, '33; Bates, Lahr and Riddle, '35).

Although the appearance of the ovary of the control lizards indicates that the pituitary is relatively inactive, and despite the fact that lizards placed under presumably favorable environmental conditions show no ovarian stimulation, nevertheless it is suggested that the results occurring from treatment with LH and FSH fractions were caused by an interaction of these substances with the animals own pituitary. This suggestion seems reasonable since it has been found in these laboratories that when normal adult rats are injected with either fraction, a very large 'mulberry' ovary with many corpora lutea is developed.

The variety of the results obtained from injections of the antagonist fraction into immature rats makes it difficult to assign any typical action to it. When tested in these laboratories it has been shown to augment the gonad-stimulating action of certain preparations if injected in one way, while under certain other conditions it inhibits such action. Evans and his co-workers ('36) concluded that the antagonist fraction inhibited the follicle-stimulating substance. As reported in this paper, it has shown ovarian stimulating properties in lizards when injected either alone or with an LH preparation

that was relatively ineffective by itself in the lizard and which had only weak luteinizing action in the rat.

Since it was found that lizards which were injected with large amounts of an unfractionated anterior pituitary extract while hibernating at a low temperature failed to respond with an increase in ovarian weight, it would seem to be necessary to have a certain minimal temperature and level of metabolism in order for gonadotropic substances to act.

It is known that the reptilian hypophysis elaborates a thyrotropic hormone (Hellbaum, '36; Schaefer, '33), that thyrotropic extracts from mammalian sources will stimulate the thyroid gland of lizards (Eggert, '36) and that the metabolic rate of the horned lizard is increased at higher temperatures (Potter and Glass, '31). In view of such findings it was thought possible that an increase in the amount of thyroxine would bring about a stimulation of the ovaries of the horned lizard.

This expectation was not realized for injections of thyroxine into animals kept at a high temperature were detrimental to the ovaries as shown by their decreased weight and atrophic appearance.

Large doses of thyroxine have been shown to have a similar effect in the rat (Hayashi, '29). Moreover, Leonard ('36) concluded from his experiments that thyroxine inhibits the action of the FSH fraction of anterior pituitary extracts injected into immature female rats.

Hypophysectomized-thyroidectomized lizards should be subjected to treatments of the type reported in this paper. Such experiments would probably yield data of value in determining the importance of the thyroid gland and the different gonadotropic fractions in controlling gonadal activity in this species.

SUMMARY

Groups of sexually inactive female horned lizards were kept for 9 days under conditions of continuous artificial lighting, a temperature of 32°C. and an oversupply of food. When they

were injected with follicle-stimulating, luteinizing and gonadotropic-inhibiting fractions of anterior pituitary extracts and with the serum of pregnant mares, a large increase in ovarian weight was produced as a result of increased yolk deposition.

Animals kept under the same conditions but injected with thyroxine had ovaries which were atrophic in appearance and which weighed less than those of controls.

Other lizards were kept in hibernation at a temperature of 5°C. Their ovaries failed to respond to treatment with an unfractionated anterior pituitary extract.

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OVOGENESIS DURING SEXUAL MATURITY. THE FIRST STAGE, MITOSIS IN THE GERMINAL EPITHELIUM, AS SHOWN BY THE COLCHICINE TECHNIQUE

EDGAR ALLEN AND R. N. CREADICK

Department of Anatomy, Yale University School of Medicine

ONE PLATE

It is always interesting to find new and more convincing evidence for a process as fundamental as the formation of new eggs during adult life. There has been little question of this in some of the seasonally breeding lower vertebrates where the evidence is clear. In mammals, however, it is more difficult to find convincing evidence; there being merely as many negative reports as positive findings. For reviews of the literature see Allen ('23), Evans and Swezy ('31) and Swezy ('33).

Recently we have reported using the alkaloid drug, colchicine, which arrests mitosis in metaphase (Dustin, '34, '35; Lits, '34 a, b; Ludford, '36), in the study of growth (hyperplasia) of genital tissues stimulated by sex hormones (Allen, Smith and Gardner, '36, '37). This technique is a splendid new tool for the study of hyperplasias, and applied in the research for formation of new eggs, it furnishes very convincing evidence.

Obviously, if new ova are formed from the germinal epithelium of the adult ovary, it is necessary that cell division occur there 1) to provide the cells which differentiate into ova (and probably also into follicle cells) and 2) to replace cells which take part in formation of follicles or 'egg tubes.' In the first instance the plane of cell division is such that one

of the daughter cells sinks into the underlying stroma and migrates toward the center of the ovary; in the second, both daughter cells may remain in the germinal epithelium. In figures 5 to 10 (Allen, '23), both planes are represented. In the above study of conditions in the ovary of the adult mouse counts of mitoses in the germinal epithelium of serially sectioned ovaries yielded totals ranging between 28 and 179 per ovary during oestrus, and very few indeed at other times in the cycle. This number shows cell division but is not impressive as an index of hyperplasia.

In a recent series of young adult mice in which the colchicine technique was used to arrest mitoses, counts ranging between 1000 and 2000 mitoses in the germinal epithelium per ovary at oestrus have been obtained. Note the frequency of mitotic figures in the limited regions shown in the illustrations. There are so many that detailed counts seem futile. Figure 4 is especially good since it shows older stages in differentiation of ova and their follicles.

The animals studied include 1) normal adult mice at oestrus, some mated, others not, and 2) puberal mice injected with anterior pituitary extract 'prephysin,' and pregnant mares' serum. When medium and large follicles are present there seems to be also an increase in cell division in the germinal epithelium. Incidentally, the sharp contrast is shown between the rapidly growing follicle filled with 'colchicine' mitoses (at lower pole of fig. 1) and atretic follicles and early corpora lutea which are relatively quiescent as far as cell division is concerned.

These photographs leave little doubt about proliferation of the germinal epithelium of the mouse ovary during sexual maturity. Further studies of variation of this process with different stages of the oestrous cycle, pregnancy and lactation are being made.

SUMMARY

Active mitosis in the germinal epithelium, the first stage of oogenesis, occurs in the ovaries of the mature mouse at oestrus. The evidence, accentuated by arrest of mitosis with colchicine, firmly establishes this fundamental process in this mammal.

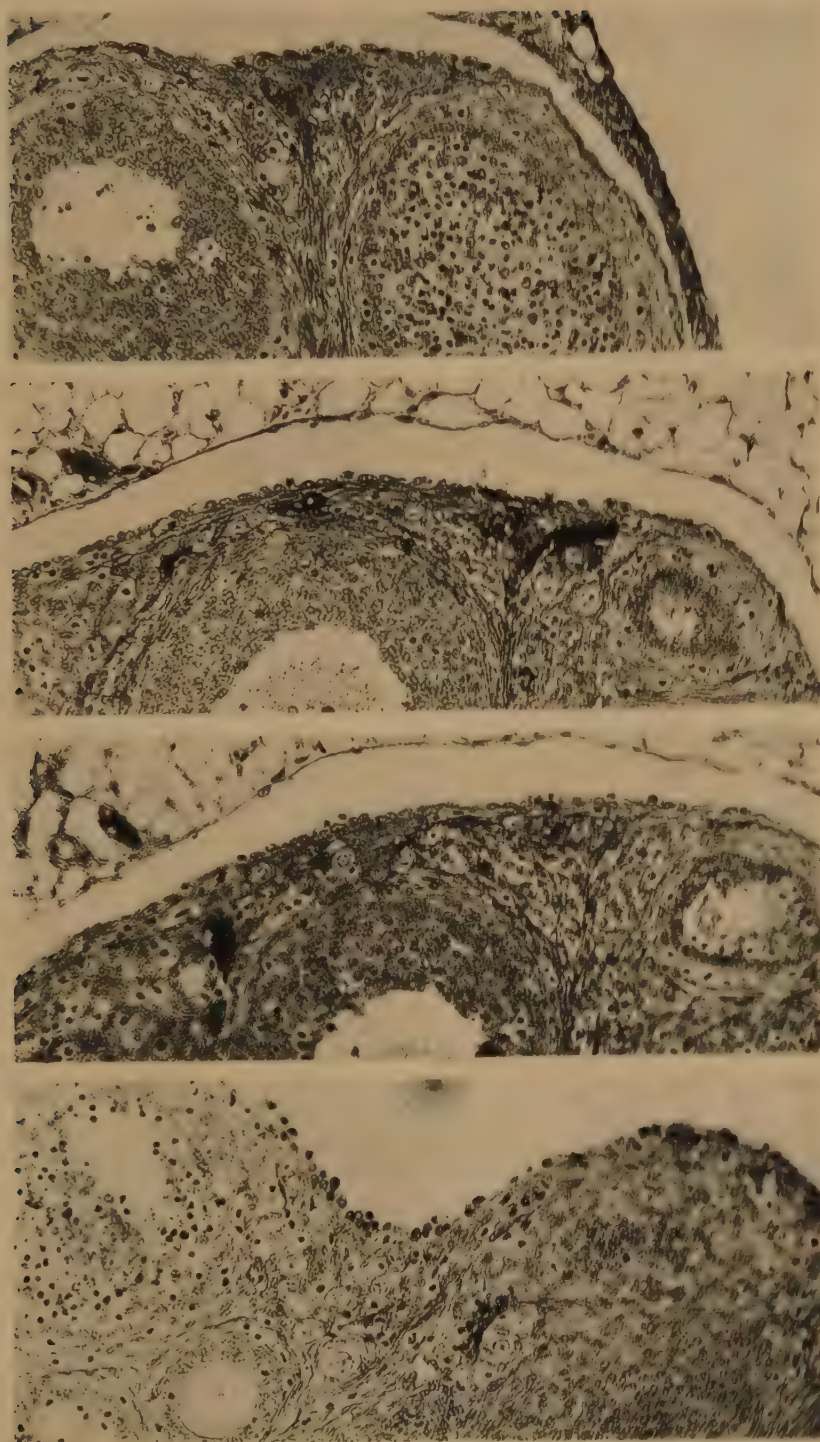
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PLATE I

EXPLANATION OF FIGURES

Formation of new ova from the germinal epithelium of the ovary in the young sexually mature mouse. $\times 240$. Mitoses arrested by colchicine, most frequent at oestrus, constitute the first stage. Later stages are also abundant.



RESPONSE OF THE RAT ENDOMETRIUM TO CANCER GRAFTS

F. E. MOHS¹ AND M. F. GUYER

School of Medicine and Department of Zoology, University of Wisconsin, Madison

FOUR FIGURES

Despite the extensive investigations which have been made on the transplantation of normal and of neoplastic tissues no attention has been given to implantation into the endometrium, the one tissue with the prime function of receiving implants, namely blastocysts. The present paper deals with the behavior of cancer implants when placed in the uterus under various conditions and with the response of the endometrium to these implants.

In a study of the effects of uterine cancer implants upon the histology of the anterior lobe of the pituitary gland Guyer and Claus ('33) observed that it was somewhat more difficult to obtain positive implants by intrauterine inoculation than by subcutaneous implantation. Subsequent microscopic studies of the positive implants revealed that in no instance was the cancer graft situated in the endometrium but that in each case the implant was growing in the myometrium or serosa. These findings suggested that the endometrium was refractory to implantation, and that takes were obtained only when the grafts were inadvertently placed in the outer layers of the uterine wall. This conclusion is here confirmed and extended.

¹ Jonathan Bowman Fellow in Cancer Research.

INTRAUTERINE INNOCULATION OF NORMALLY CYCLIC RATS

Intrauterine inoculation of cancer was accomplished by exposing through a lumbar incision the upper end of the right uterine horn, incising its upper extremity and inserting by means of a dull no. 18 trochar four fragments of cancer 0.5 mm. in diameter at various levels in the lumen. Care was exercised to avoid puncturing or traumatizing the mucosa. When extrusion of the implants seemed likely the uterine incision was closed by suture. The phase of the estrous cycle was determined by daily vaginal smears. Flexner-Jobling carcinoma was used, and in each case a control subcutaneous implant of the same size was made.

The animals were inoculated at various times during estrus and diestrus. After intervals of 2 to 25 days (average 14 days) the animals were killed and the uterus was examined grossly and microscopically for cancer. The results were as follows:

Number of rats inoculated during diestrus	17
Number of takes in endometrium	1 (5.9%)
Number of rats inoculated during estrus	11
Number of takes in endometrium	0
Number of rats with normally cyclic uteri	28
Number of takes in endometrium	1 (3.6%)

In each case the control subcutaneous implant was viable. There were positive implants in the outer layers of the uterus in three of the rats planted during diestrus and in one planted during estrus; these evidently were due to inadvertent puncturing of the mucosa with implantation external to the endometrium.

The one positive endometrial implant consisted of a very small clump of cancer cells surrounded and infiltrated with many leukocytes giving the appearance of a regressing graft. Clearly the normally cyclic endometrium is highly refractory to cancer implantation.

INTRAUTERINE INNOCULATION OF ANESTROUS UTERI

The possibility was considered that the cyclic changes in the normal endometrium might be responsible for the refractoriness of this tissue to cancer implantation. Accordingly implants were made in the uteri of anestrus rats.

Anestrus in ten rats was produced by castration at the time of implantation. In two rats anestrus was the result of toxic states due to an abscess in one and due to an orbital tumor in another. Implantation was by the technic described above except that in the castrates the implants were inserted into the lumen of the right horn through the end opened during castration. Flexner-Jobling carcinoma was used in all except one case in which the highly malignant Philadelphia I sarcoma was employed.

At intervals of 7 to 30 days (usually 21 days) the animals were killed and the uteri were examined for cancer. The results were as follows:

Number of castrate rats inoculated	10
Number of takes in endometrium	1 (10%)
Number of toxic anestrus rats inoculated	2
Number of takes in endometrium	0
Number of anestrus rats inoculated	12
Number of takes in endometrium	1 (8.3%)

The one positive implant in the endometrium was the highly malignant sarcoma. It grew very slowly, being less than 1 mm. in diameter at 14 days as compared to the 7 mm. subcutaneous graft.

These findings indicate that the anestrus endometrium is common with the normally cyclic endometrium is highly refractory to cancer implants. Hence, the cyclical changes in themselves are not responsible for the refractory nature of this tissue.

INTRAUTERINE INNOCULATION OF PSEUDOPREGNANT RATS

It has been shown by Long and Evans ('22) that rats may be kept in a corpus luteum phase similar to that of the first

part of pregnancy by sterile copulation or by artificial stimulation of the uterine cervix. This condition of pseudopregnancy is characterized by gestational changes in the organs normally affected by pregnancy. As Loeb ('07) first showed the uterus becomes sensitive for a time during pseudopregnancy so that trauma produces decidual reactions similar to those formed by the implantation of the blastocyst. The pseudopregnant state persists for only 12 to 14 days as compared to the 21-day gestation period of the rat.

Since normally cyclic and anestrous endometria had been shown to be refractory to cancer implantation the possibility was considered that the pseudopregnant endometrium, being prepared as if for nidation of the blastocyst, might be receptive to cancer implants. This proved to be the case as the following experiments show.

Experimental. The pseudopregnant state was elicited by means of artificial stimulation of the uterine cervix during proestrus or estrus. The cervix was visualized by means of a small aural speculum and a head mirror. Then the cervix was massaged with a probe until muscular contractions suggestive of an orgasm were obtained. In two rats the pseudopregnant state of the lactating rat's uterus was utilized, implants being made 4 to 7 days postpartum.

During the supervening trauma-sensitive period, 4 to 7 days after cervical stimulation, four small fragments of cancer were inserted as described in the above experiments, care being exercised to reduce the trauma to the endometrium to a minimum.

After intervals of from 1 hour to 41 days the animals were explored by laparotomy or killed and the uterine implants studied grossly and microscopically. Vaginal smears were followed daily to determine the length of the pseudopregnant period and to observe the 'placental sign.' The latter refers to red blood cells in the vaginal smear and is an indication of deciduoma formation.

Results. In marked contrast with the findings with normally cyclic and anestrous rats the endometrium of pseudo-

pregnant rats proved to be highly receptive to cancer implants as the following results show:

Number of pseudopregnant rats inoculated	20
Number of takes in endometrium	17 (85%)
Number of lactating rats inoculated	2
Number of takes in endometrium	2 (100%)

Histology. Microscopic study of thirteen cancer grafts at intervals varying from 1 hour to 23 days revealed characteristic changes in the graft and in the surrounding endometrium. At 1 hour the graft snugly filled out the lumen of the uterus. The epithelium was denuded in a few areas and in others degeneration appeared to be already under way.

At 24 hours a cleft still persisted part way around the periphery of the graft, but at some places the epithelium was obliterated allowing contact between the endometrial stroma and the graft. At the latter sites the cancer cells remained viable while elsewhere degeneration and necrosis occurred. The tendency for the graft to establish predominantly on the antimesometrial side of the uterus was already evident as illustrated in figure 1. An early but definite decidual reaction was apparent as evidenced by the typical engorgement and vacuolation of the endometrial stroma cells.

At three days the graft was growing actively into the decidual tissue. The remaining fibrous stroma of the original cancer fragment was undergoing hyalin degeneration. The new outgrowths of carcinoma had a conspicuous absence of stroma in marked contrast to the profuse stroma of Flexner-Jobling carcinoma growing in any other tissue.

By 5 days the original stroma of the graft had largely disappeared and a few decidual cells were interspersed among the cancer cells at the growing edge.

At 9 days after implantation, which corresponds with about the thirteenth day of pseudopregnancy, the decidual tissue became degenerate and was absorbed or was cast off with the onset of the first estrus. However, the cancer implant had become established so that it remained viable in spite of the absence of decidua and the return of cyclic changes.

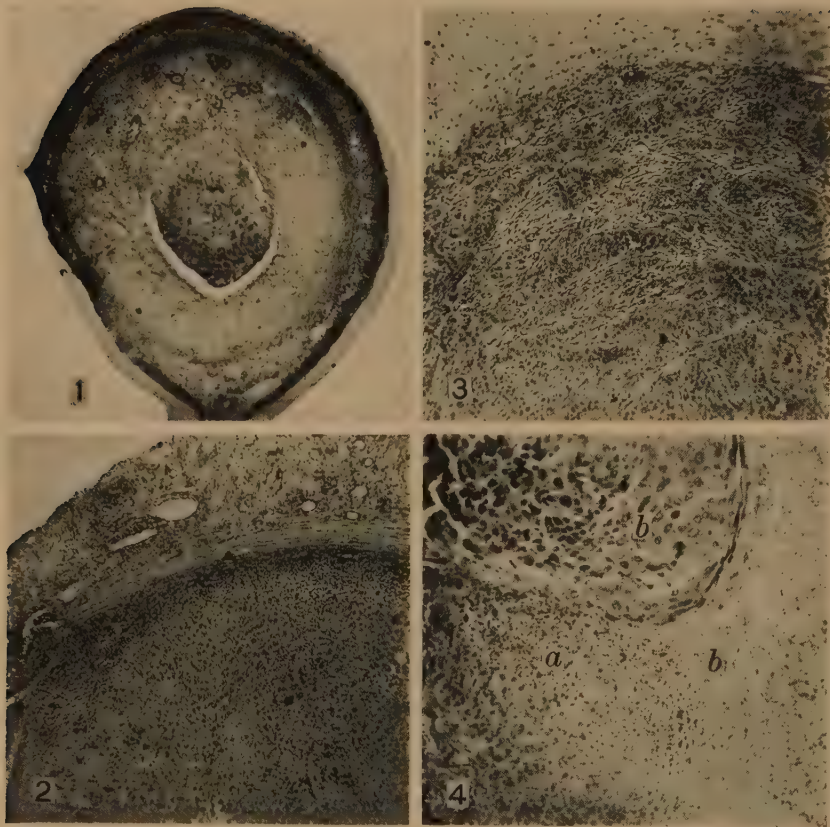


Fig. 1 A 24-hour graft in the lumen of a pseudopregnant uterus. Note the tendency to attach to the antimesometrial side of the endometrium. Except at point of attachment the cancer is necrotic as indicated by the darkly staining areas.

Fig. 2 A 9-day graft which became established in the endometrium during pseudopregnancy. The latter was terminated 2 days before this specimen was taken. Note paucity of stroma in the cancer, relatively slight necrosis, and marked distention of uterus.

Fig. 3 A 9-day subcutaneous implant of Flexner-Jobling carcinoma illustrating the usual profuse stroma.

Fig. 4 Cancer (a) growing actively amid the decidual tissue (b) of a rat ordinarily resistant to the tumor employed.

The extraordinary lack of stroma in the cancer, the paucity of central necrosis and the stretching of the wall of the cyclic uterus is illustrated in figure 2. The striking lack of stroma is accentuated by comparison with a subcutaneous implant at the same interval (fig. 3).

By 13 days the cancer showed some evidence of degeneration in the antimesometrial sector, but the main mass remained viable despite the cyclic changes in the uterus.

From 14 to 23 days the specimens in some cases showed varying degrees of inhibition of the growth of the implant. In some almost complete regression occurred while in others growth continued unrestricted with eventual erosion of the myometrium. Once the cancer broke through the endometrium the stroma of the carcinoma again became very profuse.

Comparative size of implants. The actual size of the endometrial implants was invariably less than that of the control subcutaneous implants, the ratio varying from 1:2 to 1:12 with an average of 1:4. However, this disparity was in considerable part due to the great difference in the amount of stroma in the two locations, the subcutaneous implant consisting of 30 to 60% stroma while the endometrial implants showed an almost complete absence of stroma.

Part of the slower growth of the endometrial implant may well be due to the pressure exerted by the myometrium which after 7 to 8 days is put at a considerable stretch. It was noted that once the implants broke through the myometrium their growth rate was at least as great as that of the subcutaneous implants.

In the case of the implants established during pseudopregnancy there was besides the above factors a variable degree of inhibition of the grafts upon reestablishment of the sex cycles. This inhibition apparently is due to the unfavorable nutritive capabilities of the normally cyclic endometrium.

Decidual reaction. Evidence of decidual reaction either by histological study or by the presence of the 'erythrocyte sign' in the vaginal smears was present in fifteen of seventeen rats (88.2%) bearing endometrial cancer implants. The

extent of the reaction however was usually not as great as is elicited by the developing embryo at a corresponding stage of pregnancy. That the deciduomas in the region of implants were produced by the implant and not by trauma incident to inoculation is evidenced by the fact that very rarely were deciduomas formed except at the site of the implants.

Implantation site. There was a definite tendency for implantation to occur at the antimesometrial side of the uterus. Of nineteen specimens studied eleven (58%) implanted primarily on the antimesometrial side, while five (26.3%) attached at multiple points, two (10.5%) on the mesometrial side, and one (5.2%) 90° from the mesometrium.

Length of pseudopregnant period. The growing cancer implant in the uterus had no tendency to lengthen the span of the pseudopregnant period. Thus in ten pseudopregnant rats with uterine implants the span of the pseudopregnant period varied from 11 to 16 days with an average of 12.4 days which is about the normal length of the pseudopregnant period. The reestablished cycles were normal in character despite the presence of uterine cancer implants.

To determine if a larger, more rapidly growing tumor would lead to a prolongation of pseudopregnancy a rat was inoculated with the highly malignant Philadelphia I sarcoma. An erythrocyte sign on the eleventh day of pseudopregnancy signified a decidual reaction and at 12 days the end of pseudopregnancy was indicated by an estrus smear. The tumor was allowed to grow and estrus occurred at 16, 21 and 26 days. Laparotomy at 28 days showed the uterus to be markedly distended by a sausage-shaped mass 9×24 mm. At 30 days cervical stimulation was performed while the rat was in estrus. A pseudopregnant period of 13 days ensued indicating that even a very large rapidly growing mass in the uterus is unable to prolong the pseudopregnant period to a span comparable to that of the gestation period.

TRANSCERVICAL INNOCULATION OF SUSPENSIONS OF CANCER AND
NORMAL TISSUE CELLS

The successful inoculation of the pseudopregnant uterus by the introduction of grafts suggested that the sensitized endometrium might be receptive to suspensions of cancer cells introduced by transcervical injection. Accordingly, the pseudopregnant condition was elicited by cervical stimulation and 4 to 5 days later 0.1 cc. of a suspension made by passing cancer tissue through a mincer and mixing with an equal amount of physiological saline was inoculated through the cervical os by means of a syringe and no. 18 needle.

Autopsy at the ninth to twenty-fifth day of pseudopregnancy of the thirteen rats thus inoculated revealed in no instance a positive cancer implant in the endometrium. However there was found in all the rats examined during pseudopregnancy decidual reactions varying from a single small deciduoma to 'giant' deciduomas involving the entire extent of the injected horn.

Cancer suspensions were not peculiar in this ability to elicit decidual reactions since similar deciduomas were produced by thyroid suspensions in all of twelve rats, by placental suspensions in all of five rats, and by testis suspensions in one of two rats. It made no difference whether the tissues were from cancerous or non-cancerous rats. Lesser degrees of decidual reactions were elicited by filtrates of proteolytic bacteria and even by physiological saline when injected with a little pressure to induce trauma to the endometrium.

Cancer suspensions were also injected into the uteri of two diestrus, four lactating and two pregnant rats with essentially negative results.

INTRAUTERINE INNOCULATION OF CANCER IN A STRAIN
RESISTANT TO FLEXNER-JOBLING CARCINOMA

By selective breeding a strain of rats has been bred which is almost completely resistant to implants of Flexner-Jobling carcinoma. As compared to the 85% susceptibility of the original strain 1043 rats of the selected strain showed only

5.9% takes at 3 weeks after implantation and ultimately every cancer regressed. Benzpyrene-induced sarcomas in either strain failed to grow in the other, and parabiotic union of resistant rats with susceptible rats was unsuccessful.

In spite of these evidences of marked antagonism between the tissues of the two strains interbreeding was regularly successful. The embryos which were later to be resistant rats were as easily nourished by the susceptible mother's decidua as were those which later were to be susceptible and the converse. This suggested that the decidua might be a tissue providing nourishment to tissues which otherwise would elicit an antagonistic response on the part of the maternal organism.

Cancer implantation into the decidua of the resistant rats offered a means by which this hypothesis could be tested. If cancer would grow in the decidua of rats otherwise refractory to the neoplasm the suggested 'buffering' function of the decidua would be still more strongly indicated.

Resistant rats were rendered pseudopregnant and innoculated by the usual technic. Of fourteen rats thus innoculated evidence of successful implantation was present in ten (71%). In four cases positive implants were present on microscopic sections; all of these specimens were removed during or at the end of pseudopregnancy. In one rat killed during the estrus at the termination of pseudopregnancy the implant was caught while in process of being extruded, but in the three rats killed during pseudopregnancy the implants were actively growing amid the decidual tissue which was always present around the successful grafts (fig. 4). Growth appeared definitely more active than in the subcutaneous grafts.

In six of the rats killed soon after the termination of pseudopregnancy, pits and pockets on the antimesometrial side of the endometrium marked the sites of implants which evidently had been successfully nourished until the termination of pseudopregnancy at which time they had been extruded along with the decidual tissue.

Five rats of the Flexner-Jobling-resistant strain in diestrus, one in estrus and one in toxic anestrus were implanted

with negative results in each case, again showing that the non-sensitized endometrium is refractory to cancer implantation.

DISCUSSION

These experiments establish the fact that the normally cyclic endometrium is highly refractory to implanted Flexner-Jobling rat carcinoma. Whether implantation is done during estrus or during diestrus is of little importance. The mechanism of this refractoriness is not entirely clear. It is not dependent upon the cyclic changes in the uterus per se since the anestrus uterus is also refractory.

The fact that the sensitized endometrium of the pseudo-pregnant or lactating rat's uterus is highly receptive to cancer implants indicates that the endometrium is not inherently refractory, but that the non-sensitized membrane merely lacks the vascular and nutritive capabilities required for successful implantation. From a teleological standpoint it is easy to see why the sensitized endometrium is so receptive to implantation, but it is difficult to see why the non-sensitized endometrium should be so much more refractory than any other tissue of the body to implantation.

A significant observation was the almost complete absence of the ingrowth of fibrous stroma into the cancer grafts in the endometrium. Flexner-Jobling carcinoma growing in any other site elicits a profuse stroma reaction. In several instances in which the graft had eroded through into the myometrium and serosa the part of the cancer nourished by these tissues showed heavy stroma while the part of the graft nourished by the endometrium was practically free of stroma.

In spite of this paucity of stroma the endometrial implants were remarkably well nourished as indicated by the small amount of central necrosis which is pronounced in subcutaneous implants. Evidently the decidua has a very highly developed capacity to nourish implanted tissues, and this without extensive ingrowth of fibrous tissue. The latter may be an important function of the decidua in pregnancy where ingrowths of large quantities of fibrous tissue into the

placenta would prevent placental separation at parturition. This may well be an explanation for the condition of placenta accreta in which, as recently pointed out by Tomaiuolo ('36), lack of decidua is a prominent feature. In the absence of decidua it is probable that fibrous ingrowths from the myometrium cause adherence of the placenta.

With the excellent nutrition of the graft as shown histologically it is surprising that the endometrial grafts were regularly smaller in size than subcutaneous grafts in the same rat. However, when it is considered that 30 to 60% of the volume of subcutaneous implants consists of stroma as compared to the very meager stroma of the endometrial implant it is evident that the actual number of cancer cells in the two locations is often about the same. In some of the grafts examined after reestablishment of the estrual cycle some degeneration and necrosis was seen which may have been due to the poor nutrition afforded by the normally cyclic endometrium and in the case of the larger grafts to the pressure exerted by the uterine muscle which loses its elasticity after the termination of pseudopregnancy. In some cases the grafts after becoming established during pseudopregnancy continued to grow progressively despite the cyclical changes; evidently permanent vascularization of the graft occurred.

In several respects the implantation of cancer in the sensitized endometrium of the pseudopregnant uterus resembles the nidation of the embryo in the pregnant uterus. Thus, the cancer graft like the embryo usually elicits a decidual reaction around itself. This reaction, however, is rarely as extensive as that around an embryo in a corresponding stage of pregnancy. It seems probable that the cancer graft like threading, piercing or electrical stimulation acts as a non-specific traumatism, but in view of the relatively slight trauma produced by the soft mass of cancer the amount of decidual reaction is correspondingly small. The comparatively great decidual reaction produced by the embryo apparently depends upon some more specific decidua-eliciting mechanism the exact nature of which is not yet known.

Again like the embryo the cancer graft tends to gain its nourishment at first from the antimesometrial endometrium. However, here too the analogy is not perfect since the cancer graft occasionally attaches at other points. It seems that the antimesometrial surface is the most favorable for implantation in general but that implantation on this surface occurs almost invariably only in the case of the implanting blastocyst.

The cancer implant definitely differs from the embryo in that the former lacks the ability to maintain the anestrus of pseudopregnancy to a duration approaching the normal gestation period of 21 days. The relatively small size of the cancer implants moreover do not account for this since even a 30-day implant which was at least the size of an embryo failed to prolong the pseudopregnant period. Evidently the embryo and its appendages maintain anestrus during the latter part of pregnancy by other means than by distention of the uterus by a growing mass of tissue.

This confirms the conclusions of Newton ('35) who found that estrus was prevented for the duration of gestation after removal of the embryos if the placentae were left intact, but if the placentae were necrotic estrus ensued. He therefore concluded that the mechanical stimulus of the placental mass was not alone responsible for the anestrus. On the other hand Selye ('34) found that anestrus could usually be prolonged after caesarian section by filling the uterus with paraffin and he concluded that the mechanical stimulus of distention was responsible.

Why transcervical inoculation of cancer suspensions in sensitized as well as in non-sensitized uteri gave such uniformly unsuccessful results is not clear. The loose cells apparently are not fixed in relation to one area of the endometrium for a sufficient period to allow attachment and nidation. In this respect again cancer tissue is different from the normal blastocyst which is comparable to the small clumps of cancer cells in the injected suspensions.

The marked decidual reaction produced by transcervical injection of cancer suspension was found not to be specific for

cancer because suspensions of various normal tissues from non-cancerous as well as cancerous rats produced a similar response.

The finding that the grafts are successful in the sensitized endometrium of rats of a strain normally very resistant to the cancer used suggests that the decidua may function to make implantation possible in the face of considerable antagonism between host and graft. Similarly, it is possible that the decidua may function as a buffer membrane between the maternal and fetal tissues preventing reactions which otherwise might lead to abortion whenever any appreciable differences in the chemical constitution of the tissues of the two individuals existed. As pointed out by Loeb ('30) the more embryonic a tissue is the less is its tendency to elicit antagonistic reactions. Similarly, Murphy ('26) has shown that heterografts are successful in the allantois membranes of the chick embryo. Decidual tissue, histologically at least, appears quite embryonic in nature and this attribute could theoretically explain its buffering capacity.

It cannot be definitely excluded that foreign grafts grow in the decidua merely because of the very favorable blood supply and nourishment afforded by this tissue. At any rate decidual tissue is essential for the growth of cancer grafts in resistant rats because immediately upon termination of the pseudo-pregnant period the grafts are extruded bodily from the uterus.

In relation to the findings of Murphy and Sturm ('34) that an aqueous extract of placental tissue is capable of retarding the growth of transplantable and spontaneous neoplasms, our finding that decidua is a very favorable tissue for cancer implantation indicates that in vivo, at least, this tissue shows no evidence of inhibitory function, and suggests that their cancer-inhibiting agent may come largely from the fetal rather than the maternal portion of the placenta. The possibility, however, of a high content of inhibiting agent in this tissue masked by a greater amount of stimulating factor cannot be excluded.

SUMMARY AND CONCLUSIONS

1. The endometrium of normally cyclic and anestrous rats is highly refractory to cancer implants.

2. The sensitized endometrium of pseudopregnant or lactating rats is highly receptive to cancer grafts.

3. The excellent nutrition of the cancer implants in the sensitized endometrium in spite of a peculiar lack of stroma growth into the graft indicates that this membrane has a characteristic highly developed nutritive function.

4. The smaller size of the endometrial implants as compared to control subcutaneous implants is in large part accounted for by the lack of stroma ingrowth. Grafts established during pseudopregnancy are retarded with the return of the normal estrus cycles due in part to pressure exerted by the myometrium and in part to the poor nutritive capacity of the cyclic endometrium.

5. Like the implanting blastocyst, the implanting cancer graft elicits a decidual reaction which, however, is less marked than that produced by the embryo, the latter probably possessing a specific decidua-producing mechanism.

6. Like the embryo, but not so invariably, the cancer implant tends to attach first at the antimesometrial surface of the endometrium.

7. Unlike the normal conceptus the endometrial cancer graft regardless of size is unable to maintain the anestrous period of pseudopregnancy to a duration approaching that of gestation. Therefore, more than the mechanical effect of a growing mass in the uterus is necessary to maintain the anestrus of the latter part of pregnancy.

8. Transcervical inoculation of cancer suspensions gave no positive implants but did produce marked decidual reactions. The latter were not specific for cancer since various normal tissues had the same effect.

9. In a strain of rats highly resistant to the cancer used, endometrial implants grew actively until the termination of the pseudopregnant period when they were extruded. A buffering function of the decidua preventing antagonistic reactions between the host and somewhat foreign grafts or embryos is suggested.

10. In view of the excellent growth of cancer in decidual tissue it is suggested that the cancer-inhibiting agents obtained by certain workers from placental tissue many come largely from the fetal part of the placenta rather than from the maternal part.

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HYPOPHYSECTOMY AND ITS EFFECTS ON MALE REPRODUCTIVE ORGANS IN A WILD MAMMAL WITH ANNUAL RUT (CITELLUS)¹

L. J. WELLS AND E. T. GOMEZ²

*Department of Anatomy and Department of Dairy Husbandry, University
of Missouri*

TWO TEXT FIGURES AND ONE PLATE (EIGHT FIGURES)

INTRODUCTION

The effects of hypophysectomy on reproductive organs of male mammals with an annual sexual cycle have not previously been investigated, to our knowledge. The senior author has for several years centered his attention upon reproductive organs of the male ground squirrel (*Citellus tridecemlineatus*) with the hope that some knowledge could be gained concerning the factors responsible for cyclic sexual activity (Moore et al., '34; Wells, '34, '35 a, '35 b; Wells and Moore, '36; Zalesky and Wells, '37). These studies indicated that the anterior pituitary gland is definitely related to the annual sexual cycle in the male, and this observation was supported by results of gross and microscopical studies of the pituitary gland (Nelson, '36). The object of the present investigation was to determine the character of sexual activity in males experimentally deprived of their pituitary glands. The present paper is the first of a series of reports concerning hypophysectomized ground squirrels, and the effects elicited by various hormonal agents in hypophysectomized males will be considered later by the writers. We wish to thank Dr.

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²Contribution from the Missouri Agricultural Experiment Station, Journal Series no. 521.

Carl R. Moore, Dr. M. D. Overholser and Dr. C. W. Turner who have critically read the manuscript of this paper; also, Miss Helen Z. Hunt who drew text figures 1 and 2.

METHODS

Seventy operated males and twenty-eight untreated controls were employed in this study, yet reproductive organs from only thirty-one operated and nine control animals were preserved for detailed study. Early postoperative mortality was high (56%); however, thirty experimental animals lived until sacrifice, occurring 3 to 5 months after operation, and only one male reported in table 1 died previous to the date planned by us. Experimental and untreated males were kept in adjacent wire cages placed in a steam heated and skylighted laboratory room. Their diet consisted of mixed grain, Purina dog chow, fresh lettuce, whole milk, salt and water.

The surgical technique finally adopted by us, essentially a modification of the method of Smith and White ('31) and White ('33), involved an oral approach to the pituitary. The animal, following access to unlimited food and water, was given an intraperitoneal injection of nembutal in sufficient quantity to induce about 80% narcosis for a period of about 2 hours. Sub-lethal doses that prolonged narcosis for a period longer than 2 hours greatly increased the hazards of postoperative recovery. The animal was placed on its back on the operating board (fig. 1), and complete surgical anesthesia was secured by the cautious periodic use of small amounts of ether. The physiological effects of nembutal on blood pressure, and the use of only small quantities of ether, tended to reduce the frequency and severity of hemorrhage. The operating board was then inclined about 20° from the horizontal, so that the animal's head was lower than the rest of the body. This procedure was adopted in order to keep the amount of hemorrhagic blood, flowing by gravity to the larynx, at a minimum. The mouth was held open by rubber bands, which were looped around upper and lower incisors and attached to

TABLE 1

Effects of hypophysectomy on reproductive organs. Data expressed as averages for each group

GROUP	NUMBER OF ANIMALS	MONTH KILLED	SIZE OF PITUITARY FRAGMENTS, ESTI- MATED PER CENT OF TOTAL HYPOPHYSIS	BODY WEIGHT IN GRAMS	TESTIS		SEMINAL VESICLES		PROSTATE GLAND		COWPER'S GLANDS		BULBAR GLAND		EPIDIDYMS		
					Weight in grams	Quantity of spermatozoa *	Weight in grams	Epithelial height in micra	Weight in grams	Epithelial height in micra	Weight in grams	Epithelial height in micra	Weight in grams	Epithelial height in micra	Weight in grams	Epithelial height in micra	Quantity of spermatozoa *
Animals operated after producing spermatozoa, for periods of 25 to 26 days, and killed during the breeding season																	
A	4	May	6.3	150	0.1252	0	0.0478	15.5	0.0228	7.3	0.0569	8.1	0.1489	7.9	16.6	0	25.2
B	2	May	Unoperated	195	0.6550	4	1.4473	27.9	0.2099	17.8	0.4095	16.8	0.7504	21.0	26.8	4	19.3
Operated during aspermatic period, for periods of 3 to 5 months, and killed at times when unoperated controls had produced spermatozoa																	
C ¹	1	Jan.	²	110	0.1450	0	0.0066		0.0018	8.4	0.0020	8.4	0.0176	9.5	16.8	0	21.8
D	1	Jan.	Unoperated	235	1.0760	4	0.0770	27.4	0.0400	12.6	0.0640	18.9	0.1434	16.8	47.0	1	41.1
E	2	Feb.	7.5	149	0.1400	0	0.0091	16.6	0.0027	8.4	0.0054	8.4	0.0251	7.3	21.0	0	17.8
F	1	Feb.	Unoperated	200	0.8562	4	0.4364	37.8	0.1300	16.8	0.2331	16.8	0.4246	16.8	33.6	4	25.2
G	6	Mar.	9.0	178	0.6337	3	0.1340	22.1	0.0543	13.1	0.0919	16.0	0.1358	13.4	34.8	3	32.7
H	9	Mar.	9.7	190	0.1354	0	0.0072	11.9	0.0029	9.1	0.0057	9.2	0.0225	8.1	21.2	0	20.9
I ³	1	Mar.	12.2	127	0.1120	0	0.0200	8.4	0.0082	6.3	0.0160	8.4	0.1260	8.4	10.5	0	12.6
J ⁵	2	Mar.	0	165	0.8280	4	0.0944	27.3	0.0345	12.6	0.0681	17.8	0.2425	14.7	43.0	1	26.2
K	4	Mar.	0	157	0.1527	0	0.0066	10.5	0.0029	8.4	0.0053	8.4	0.0242	8.4	17.4	0	20.2
L	5	Mar.	Unoperated	187	0.6850	4	0.5068	35.9	0.1177	16.9	0.2064	18.9	0.4715	18.3	37.8	4	31.3

¹ Died less than 12 hours preceding autopsy.² Small fragment macroscopically detected. No microscopical study made.³ Adult with normal involution of reproductive organs at time of hypophysectomy (November).⁴ Estimated number expressed in categories ranging from 0 (none) to 4 (maximum for this species).⁵ Each animal received no post-operative treatment for 4 months and then received two rat pituitaries daily for 30 days preceding autopsy.

the board. The tongue was kept outside the surgical field by mild tension of a rubber band against the lower incisors. The soft palate was bisected inferiosuperiorly as far as the bony palate by means of a scalpel, and the bisected halves were held in lateral positions by slight tension of rubber bands on blunt wire retractors. Adrenaline chloride was used as a hemostatic agent, when needed, by applying cotton plugs soaked in the solution directly upon the source of hemorrhage. A circular hole about 1.5 mm. in diameter was bored through the sphenoid bone, opposite and ventral to the pituitary gland, by means of a dental drill (fig. 2). The dural sheath was thus

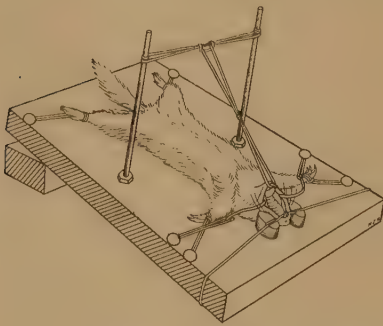


Fig. 1 Diagram showing animal anchored to operating board and prepared for pituitary removal. $\times \frac{1}{10}$.

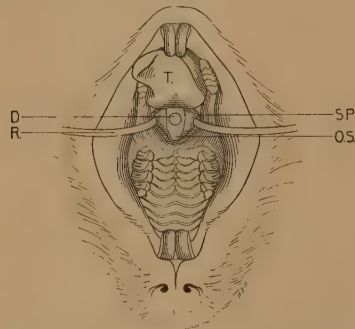


Fig. 2 Diagram showing the oral approach used in pituitary removal. D, drill hole in sphenoid bone; O.S., os sphenoidale; R., retractor; S.P., cut edge of soft palate; T., tongue. $\times 1$.

exposed, and its ventral wall was slit with a sharp dissecting needle. The entire pituitary gland, exclusive of the stalk, was removed through a glass canula by negative pressure; bleeding at this stage was rarely more than slight, but it could be controlled by direct application of adrenaline chloride. One of us watched the fragments of pituitary enter the canula, and estimated the amount of the gland actually removed. Since the surgical field was not a blind one, we could usually tell, by direct observation, the approximate amount of the gland removed. The stalk was occasionally drawn into the opening of the canula, but the magnitude of negative pressure was

kept at a level low enough to prevent destruction of the stalk and injury to the diaphragma sella. The bisected soft palate was then replaced to its normal position without the aid of stitches, and the animal was removed from the operating board.

Much post-operative care was required to bring the operated animal through early crises. While still in a state of narcosis, the animal was kept with its head higher than the rest of the body, so that blood and other fluids oozing into the nasopharynx would not drain into the larynx. If the animal succeeded in living for 48 hours after the operation, it was likely to live for a period of months. Early post-operative mortality was preceded by symptoms suggesting hypoglycemia, which has been reported in hypophysectomized mammals, particularly when the animals were in a fasting state (Braier, '31; Ré, '31; D'Amour and Keller, '33; Fujimoto, '32; Corkill, Marks and White, '33; Smith, Dotti, Tynedale and Engle, '36; Shapiro and Pincus, '36). As a precautionary measure to prevent the occurrence of hypoglycemic crises, dextrose was injected subcutaneously twice daily for the first 2 days and supplied in the drinking water for at least 1 week. When hypoglycemia had already developed in spite of our precautions, an extra subcutaneous injection of dextrose usually relieved the symptoms.

Both operated and untreated males were examined at intervals of about 2 weeks, for the purpose of determining the macroscopic condition of their reproductive organs. Testes, epididymides and bulbar gland were examined by manual palpation, and the presence or absence of scrotal pigment was observed.

At autopsy, the following procedures were used in a routine manner. The animal was killed with an overdose of chloroform, and weighed. Reproductive organs were removed, weighed while fresh, and fixed in Bouin's picro-acetic-formol solution. A smear of fresh epididymis in physiological saline solution was examined for spermatozoa. The brain was cautiously removed, care being taken to prevent a destruction of the dorsal wall of the dural sheath. The sella turcica was

macroscopically observed for the purpose of determining whether pituitary fragments existed. Regardless of the results of these macroscopic observations, the sphenoid bone was carefully removed without disturbing the sella region and fixed in Bouin's solution. Following fixation, the sphenoid bone was decalcified in either Müller's fluid or 5% nitric acid in alcohol. Serial sections of the sphenoid region were cut at 10 μ , stained with hematoxylin and eosin, and examined microscopically for pituitary fragments.

The following method for estimating the quantity of pituitary tissue in pituitary fragments was used; this method was suggested to the writers by Dr. R. T. Hill of Yale University, in a personal communication (see also Gomez, Turner, Gardner and Hill, '37). In serial sections of the sella, the largest section of pituitary tissue was measured with the aid of an ocular micrometer; two measurements of the diameter of this section, at right angles to each other, were made. These two diameters were then reduced to millimeters. The third dimension was determined in millimeters by counting the total number of the 10- μ sections containing pituitary tissue. Estimated volume of the fragment in cubic millimeters was then determined by multiplying the three dimensions. The same procedure was followed in estimating the total volume of the entire hypophysis of a normal male. Estimated size of the fragment was then expressed as per cent of the total hypophysis. This method gives no more than an approximate estimate of the size of the fragment. It is probable, however, that the fragments actually contained less pituitary tissue than is indicated by the estimates, and it seems rather certain that the fragments had no more than the estimated volume.

Six micra sections of reproductive organs from each of the forty autopsied animals were stained with hematoxylin and eosin, and studied. Sections of the testis were examined, in order to determine the character of the germinal epithelium. The qualitative character of secondary sexual organs was microscopically studied and tabulated, while the epithelial height was measured with an ocular micrometer. Five ocular

measurements of the epithelium were made, care being taken to avoid areas cut tangentially, and an average of the five readings was recorded.

RESULTS

Reproductive organs from normal males of this species have been studied previously by one of us at monthly intervals during five complete annual cycles. This experience has made possible the judicious planning of operation and autopsy dates at crucial stages in the sexual cycle.

Hypophysectomy performed during the breeding season

Four animals hypophysectomized soon after collection from the field in early April survived long enough to be of interest. One testis of each was removed on the day of hypophysectomy and weighed, average testis weight for the group being 0.8175 gm. Epididymis of each contained many spermatozoa which were motile in saline solution. After periods varying from 25 to 36 days, these males were killed. Pituitary fragments were detectable only in microscopic sections but were present in each animal. The estimated size of fragments ranged from 0.2% to 17.0%, the average for this group being 6.3% (group A, table 1).

The remaining testis of each had undergone regression and was found to be small, flabby and abdominal in position. Average weight of testes from operated males was 19.1% as great as the average weight for unoperated controls (groups A and B). Testes from operated males contained neither spermatids nor spermatozoa, while those from controls exhibited many (figs. 3 and 4).

Accessory reproductive organs of all operated animals were found at autopsy to have undergone pronounced regression. Both gross and microscopical studies indicated that these organs were essentially similar to those from castrated ground squirrels (Wells, '35 a). The prostate gland normally stores no secretion in this species, and for this reason it is perhaps the best indicator of the functional status of the accessory

reproductive system. Average prostate weight for hypophysectomized males in group A was 10.8% of that for unoperated controls in group B. Average height of prostatic epithelium in group A was only 41.0% as great as that in group B. These quantitative differences are more strikingly demonstrated by organs that store secretion. For example, average weight of seminal vesicles in the control group B was 30.4 times greater than that in the hypophysectomized group A.

Hypophysis removal during aspermatic period

Twenty-three males hypophysectomized during October and November lived long enough to qualify for our present consideration, i.e., they survived for 3- to 5-month periods until sacrifice which occurred at a time when each of seven unoperated controls showed spermatozoa. Only four of the twenty-three possessed no trace of pituitary tissue, and the other nineteen exhibited small pituitary fragments of varying size.

1. *Adults with involuted reproductive organs.* Only one of six operated adults survived long enough for our consideration. This animal was hypophysectomized on November 22nd, at a time when manual palpation indicated testes to be abdominal in position and accessory organs non-functional. Following a period of 103 days, the male was killed on March 5th. A pituitary fragment estimated to be no more than 12.2% of the total hypophysis was found in serial sections (group I, table 1).

Reproductive organs of this hypophysectomized adult failed to undergo renewed development at the season when it occurred in unoperated males (groups I and L). The testis was small, flabby and abdominal in position, and contained neither spermatids nor spermatozoa (figs. 10 and 9). Testis weight was only 16.3% of the average weight of testes from five unoperated controls. Accessory organs like-wise failed to undergo normal development, remaining in a non-functional condition.

2. *Immature males.* Twenty-two animals operated in October and November, after attaining an age of 5 to 6 months and a body weight equivalent to that of mature males, survived long enough (89 to 155 days) to qualify for consideration.

No pituitary fragments were detectable in four males. In each of these animals the entire reproductive tract was decidedly immature, while that of unoperated controls was either at a stage of maximal development (testes) or approaching a functional peak (accessory organs). These differences are represented in table 1, groups K and L, but the microscopic character of the testes should be mentioned; testes of these hypophysectomized animals invariably showed spermatogonia and spermatocytes with apparently normal morphology, but they contained no spermatids or spermatozoa (figs. 5 and 9). Reproductive organs from these four hypophysectomized males scarcely, if at all, were distinguishable from those of normal males killed at the time of hypophysectomy. Therefore, our data suggest that hypophysectomy resulted in no actual regression of reproductive organs, but caused them to remain in the same infantile condition prevailing at the time of operation.

Males possessing pituitary fragments. There were eighteen animals of this type. On the basis of sexual development, they fall logically into the two following classes, 1) those failing to produce spermatozoa, and 2) those producing spermatozoa. Reproductive organs from twelve of these animals (66.6%) resembled, in the main, those from animals with no detectable pituitary tissue. Testes failed to descend to the scrotum, remained small and contained neither spermatids nor spermatozoa (figs. 8 and 9), while accessory organs remained infantile. Detailed observations are presented in table 1, and reproductive organs from these hypophysectomized males may be compared with those from unoperated animals in the appropriate control groups as follows: groups C and D; E and F; H and L. Group averages for the estimated amount of pituitary tissue were 7.5% and 9.7%, but estimates for individual animals varied from 2.1% to 21.7%.

In the other six animals (33.3%), testes descended into the scrotum, attained a large size and produced spermatozoa which were revealed also in epididymis smears (compare groups G and L) (fig. 7). Accessory organs did not attain a breeding condition, but they showed gross and histological signs of stimulation by male hormone. Estimated amounts of pituitary tissue for individual animals varied from 2.3% to 20.1%.

3. *Implantation of fresh rat hypophyses.* Four of the male ground squirrels which were hypophysectomized while immature during November were selected for this experiment because they showed signs of being completely hypophysectomized. On February 15th when untreated controls had large scrotal testes, these four males had abdominal testes and for this and other reasons were thought to possess no pituitary tissue. On this date, one testis was removed from each; no spermatozoa were found upon microscopical study of sections from these testes. Each animal received one rat anterior pituitary daily for 30 days. The anterior lobe was hashed with scissors, suspended in a minimum amount of sterile saline solution and injected intramuscularly in the legs of the recipient ground squirrel. On the day following the last implantation, March 16th, the ground squirrels were killed. Two of them showing marked stimulation of all reproductive organs were found to actually possess hypophysis fragments of their own and therefore are not listed in table 1, however, the other two showed no histologically detectable pituitary fragments in their sellae.

In table 1, reproductive organs from the two completely hypophysectomized males (group J) may be compared with those of groups K and L in which the animals received no pituitary implants. It will be seen that the males receiving rat pituitary implants had large testes with spermatozoa (fig. 6) and accessory organs exhibiting signs of stimulation by testicular hormone; yet, these accessory organs definitely did not attain a functional breeding level.

DISCUSSION

In this study, we have experimentally removed the hypophysis from males of an annually-breeding wild rodent to see the effects on reproductive organs. The surgical approach was such that the operation was not a blind one and the pituitary stalk was left intact. We have checked the success of the operation by a study of serial sections of the sellae, and estimated the maximal amount of pituitary tissue possible in cases of incomplete removal. It is probable that many of our incompletely hypophysectomized animals actually had less pituitary tissue than the amount estimated.

Results of our experiments indicate that sexual activity in the male ground squirrel is prevented by the absence of the pituitary gland, and that it either may or may not be prevented by incomplete hypophysectomy.

The animals with incomplete hypophysectomy are of particular interest, since previously it had been found (Wells, '35 a, and unpublished observations) that the intact pituitary from males of this species has little gonadotropic potency during the non-rutting period; it is certainly noteworthy that six immature males (33.3%) were able to produce spermatozoa, even though rough estimates indicated that they had no more than from 2.3% to 20.1% of the entire pituitary gland at autopsy. The above observation makes us feel that the presence of tiny fragments of anterior lobe tissue should not be considered of little import when interpreting results of experiments in which hypophysectomized mammalian males are the test animals. It is true that 66.6% of the immature incompletely hypophysectomized males did not undergo sexual development, but other males with pituitary fragments as small as in these did.

Our data also show that fresh rat hypophyses may induce spermatozoa formation and a release of enough testicular hormone to cause stimulation of accessory organs in immature animals entirely deprived of their own hypophyses (two cases). This has also been accomplished in the intact immature male (Wells and Moore, '36).

SUMMARY AND CONCLUSIONS

Sexual development was prevented by complete hypophysectomy in immature male ground squirrels (*Citellus tridecemlineatus*), and either was or was not prevented in immature animals following incomplete hypophysis removal. It is suggested that the presence of tiny pituitary fragments should not be considered too lightly when interpreting results of experiments in which the hypophysectomized mammalian male is the test animal. Incomplete removal during the non-rutting period prevented a renewal of sexual development in one adult. Hypophysectomy for periods of approximately 1 month during the breeding season rendered the reproductive organs atrophic. Daily implantation of fresh rat hypophyses induced spermatozoa formation in two completely hypophysectomized immature males.

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PLATE 1

EXPLANATION OF FIGURES

(All sections cut at 6μ and magnified $\times 160$)

3 Normal testis surgically removed from animal 1164 on March 30th. Spermatozoa present.

4 Remaining testis of animal 1164 removed at autopsy on May 5th, following a 36-day period of hypophysectomy. No spermatids or spermatozoa were found. Normal males at this time of the year have large and spermiatic testes.

5 Testis of animal 1112 which was totally hypophysectomized for 4 months and killed on March 16th. No spermatids or spermatozoa.

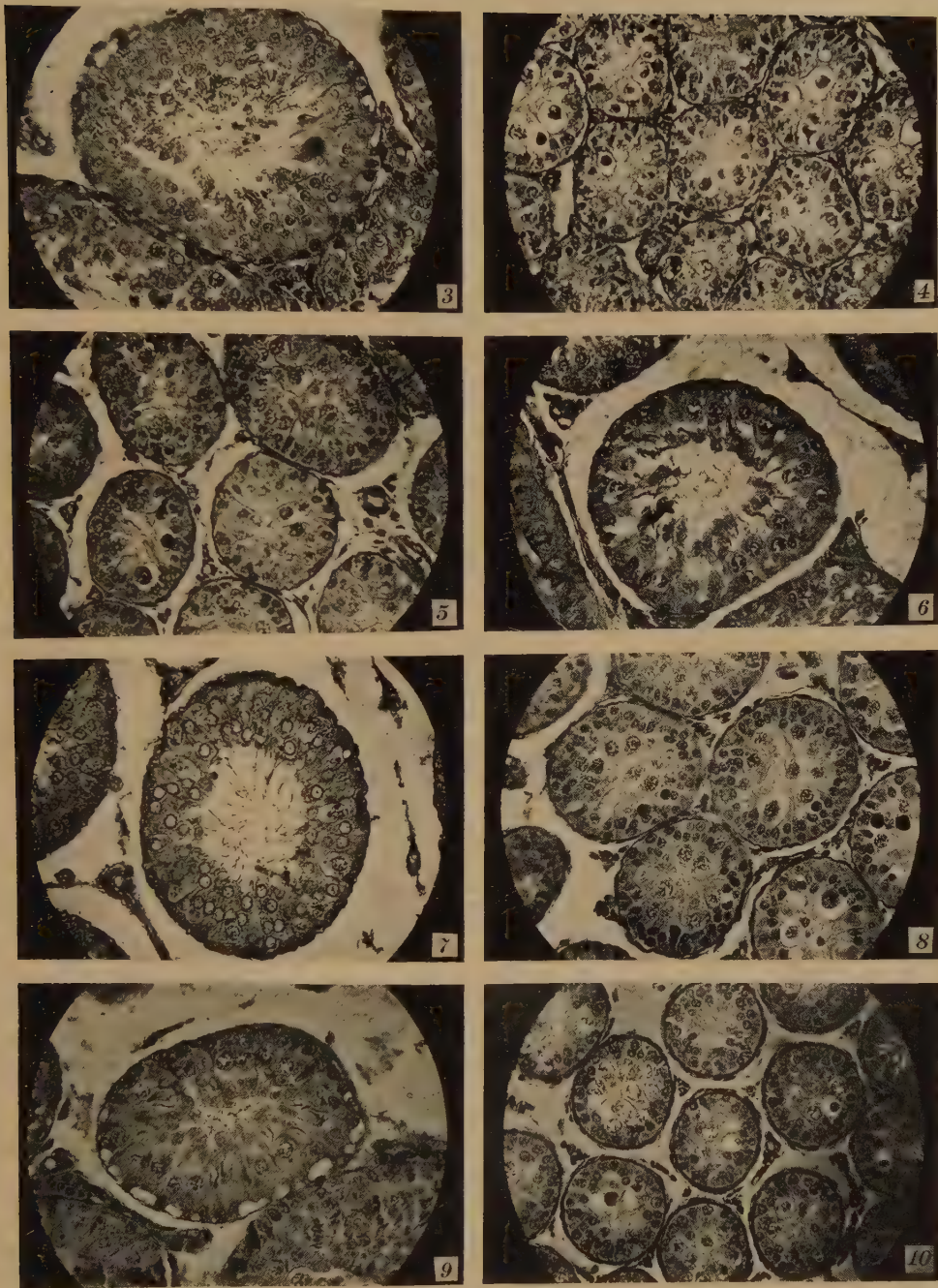
6 Testis of animal 1107 which was completely hypophysectomized for 4 months. This animal received one rat pituitary daily for 30 days preceding autopsy on March 16th. Spermatozoa present.

7 Portion of testis from animal 1113 which was hypophysectomized for 4 months and killed on March 16th. A small pituitary fragment estimated to be no more than 5.3% of the entire hypophysis was found. Spermatozoa present.

8 Testis of animal 1114 which was hypophysectomized for 4 months and killed on March 16th. A pituitary fragment estimated to be no more than 10.9% of the entire hypophysis was found. No spermatids or spermatozoa.

9 Testis of a normal male killed March 5th. Spermatozoa present.

10 Testis of animal 1122 which was an adult with involuted reproductive organs when hypophysectomized on November 22nd. Animal killed on March 5th. A pituitary fragment estimated to be no more than 12.2% of the total hypophysis was found. No spermatids or spermatozoa.



STRUCTURAL AND FUNCTIONAL RECONSTITUTION OF ULTRACENTRIFUGED RAT ADRENAL CELLS IN AUTOPLASTIC GRAFTS

ERNST J. DORNFELD

Department of Zoölogy, University of Wisconsin

TWO TEXT FIGURES AND TWO PLATES (TWELVE FIGURES)

Since the introduction of the air-driven ultracentrifuge into cytology by H. W. Beams and R. L. King in 1934, a wide variety of tissue cells have been investigated by this method. Several workers have sought therewith to establish the relative specific gravities and the physical autonomy of various nuclear and cytoplasmic components, and the results thus far have been in rather striking agreement. The validity of the conclusions, however, depends to a great extent on whether or not the components undergo post-mortem change during the process; whether cell parts after long exposure to a centrifugal force as great as 400,000 gravity units still represent essentially unchanged materials. The present study was undertaken with this problem in mind and also in consideration of the general biological question of whether specialized tissue cells can retain or regain their viability and functional capacity after their inner morphology has been severely disturbed.

The writer is deeply obligated to Prof. M. F. Guyer for the interest he has shown throughout the progress of this work as well as for his valuable counsel and criticism. In the operative manipulations and other technical procedures able assistance has been given by Richard C. Shannon and Franklin W. Kapke, to both of whom special indebtedness is acknowledged.

MATERIALS AND METHODS

For the purposes of this study a total of seventy-five albino rats were selected, males and females in about equal numbers, of well-inbred stock, and between 33 and 67 days of age. These were arbitrarily divided into two groups, forty-six constituting the transplant series, twenty-nine the control series. The transplant series was dealt with as follows.

Double adrenalectomy was performed in a 5- to 10-minute operation, using ether anesthesia. The operative technique was in the main that described by Grollman ('36, p. 252), especial care being taken to avoid any injury of the glands and to include also the surrounding connective tissue which often carries accessories. The glands, as soon as removed, were carefully cleared of the fat and connective tissue and transferred to an ultracentrifuge rotor containing mammalian Locke-Lewis solution previously warmed to 39°C. (The parts of the ultracentrifuge used are pictured and described by Beams, Gatenby and Muliyil, '36.) After centrifuging for 30 minutes at a force of 400,000 times gravity—during which period maximum stratification is obtained, as described by the writer in an earlier paper (Dornfeld, '36)—the glands were immediately taken out of the rotor, carefully halved with a very sharp scalpel, and two or three pieces autoplastically grafted into the abdominal muscles. The muscle fasciae were punctured at convenient loci with the points of scissors, and by introducing the prongs of fine curved forceps, pockets were made by wedging them apart. The halved glands could be easily introduced without damage, and the punctures were closed with single sutures of black silk marking thread.

The rats of the control series were adrenalectomized in the same manner, but no transplants made. Both groups were then given Locke-Lewis salt solution in lieu of regular drinking water over a period of about 30 days, in the expectation that the survival period might be prolonged, thereby giving the grafts a good chance to 'take.' The control rats were used to determine this survival period.

At varying intervals between 1 day and 4 months implants were removed and fixed for serial sectioning in Bouin's fluid, Bensley's formalin-bichromate-sublimate, and Sevringhaus' modification of Champy's fluid. At autopsy the original adrenal sites were removed for serial sectioning and carefully examined for accessory cortical tissue. To test the functional capacity of the grafts older implants were excised under anesthesia and the subsequent survival periods studied. Daily weight records were also kept for animals in both series.

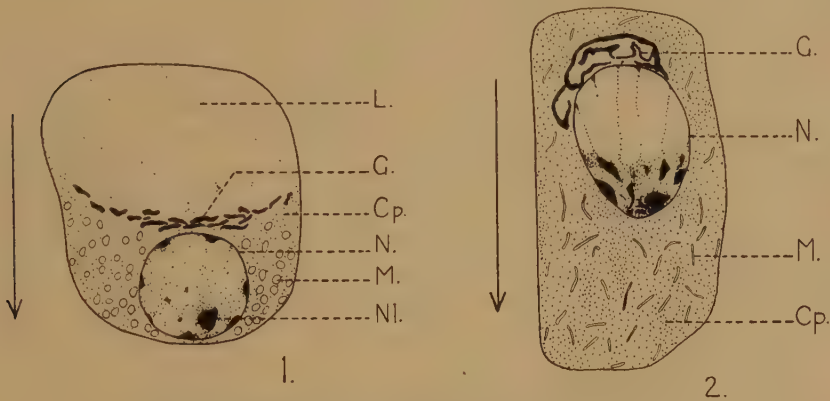


Fig. 1 Diagram of ultracentrifuged cortical cell

Fig. 2 Diagram of ultracentrifuged medullary cell.

In order to study the earliest cell changes following centrifuging, some glands were transferred from the rotor to a fresh supply of warm Locke-Lewis solution and placed in an incubator at 39°C. for varying lengths of time up to 3 hours. These specimens were then fixed and stained in the same manner as the transplants.

OBSERVATIONS

The nuclear and cytoplasmic changes produced in adrenal cells by $\frac{1}{2}$ hour of ultracentrifuging have been dealt with in an earlier paper (Dornfeld, '36). Figures 1 and 2 summarize in diagrammatic form the principal results. Figure 1, representing the changes in cortical cells, shows that the nucleus as

a whole (N.) and its contained chromatin and karyosome (Nl.) move centrifugally. The lipoidal droplets (L.) are displaced in the opposite direction, forming a single mass at the centripetal end. The Golgi apparatus (G.) comes to lie just beneath the lipoid, and the mitochondria (M.) lie embedded in the homogeneous cytoplasm (Cp.). The corresponding changes in medullary cells are shown in figure 2. Here the cytoplasm is more viscous and its components stratify less markedly. However, the nucleus as a whole tends to elongate and its chromatin becomes strongly displaced centrifugally, leaving only fine filaments extending across the karyoplasm to the nuclear membrane on the opposite side.

Early recovery changes in Locke-Lewis solution. Centrifuged glands kept in isotonic Locke-Lewis solution at 39°C. for 3 hours show marked changes in the appearance of their cells (figs. 5 and 6). The nuclei tend to return to a more central position and to regain their normal spheroid shape. The chromatin, which in medullary cells had been strongly displaced, assumes more or less its original distribution. However, the lipoid material of cortical cells remains at the centripetal side where it commonly rounds up to form one or several large globules. The position of the Golgi apparatus at the inner margin of the lipoid is not materially altered; nor is that of the mitochondria within the denser homogeneous cytoplasm.

Transplant phenomena. Examination of transplants of the first 2 weeks reveals host reactions characteristic of autoplasmic grafts. By the method of implanting halved glands, cortical and medullary cells are equally exposed to the host tissues.

In 24-hour grafts the muscle and connective tissue around the implant is seen to be edematous and infiltrated with numerous neutrophils and a small number of lymphocytes and fibroblasts. The neutrophils have invaded the implant from the periphery, this invasion being most pronounced on the second day (fig. 3). A more or less well-defined leucocytic band is thereby demarcated, within which the adrenal cells

present an appearance similar to that observed in saline after 3 hours. Particularly noteworthy is the even distribution of chromatin in medullary nuclei.

Meanwhile fibroblasts have become more abundant around the graft and during the next 3 or 4 days they are seen penetrating the implant together with the neutrophiles. Macroscopically the pink color of the graft and the appearance of blood vessels leading to it indicate the beginning of vascularization. In sections a small number of arterioles and venules may be seen closely appressed to the implant, and occasional capillaries lined by endothelial cells and carrying red cells, lymphocytes, and neutrophiles enter, sometimes deeply, into the tissue. Such capillaries become progressively more abundant, particularly in the outer region of the graft. In their neighborhood some of the cortical adrenal cells show indications of renewed activity: a few mitoses are seen, small lipoidal droplets and mitochondria are evenly distributed through the cytoplasm, and a condensed Golgi mass lies next the nucleus though no longer showing signs of centripetal polarization.

By the end of a week the neutrophiles are concentrated in the deeper portions, where they apparently aid in the destruction and removal of necrotic adrenal cells which in this region have for the most part lost their staining capacity and appear to be disintegrating. Fibroblasts now form the conspicuous host cell elements in the outer and middle parts of the transplant (fig. 4).

During the second week notable advances in the cytological reconstitution of the cortical adrenal cells take place. The entire graft becomes permeated by a loose stroma of fibroblasts and by capillaries carrying circulating erythrocytes. In the regions where the blood supply is densest the adrenal cells take on a normal appearance. Though their lipid content, as judged by visible droplets, is still small, the cells are large and possess well-rounded nuclei. Most noteworthy is the appearance of the Golgi apparatus (figs. 11 and 12). This structure is no longer a compact mass lying against one side of the

nucleus, but forms a loose encircling network, and in a few cells spreads considerably through the cytoplasm. These cells, it must be emphasized, are a part of the fasciculate layer, which in normal intact glands ordinarily show no such configuration of the Golgi material.

By the fourteenth day all implants examined show large areas of reconstituted cortical cells (fig. 7) which apparently are increased as the vascularization develops. Concomitantly mitoses become more frequent and regenerative growth becomes an important factor in the further development of the grafts. At this time medullary cells are still present, but their cytoplasm is highly degenerate, though areas of fully reconstituted cortical tissue may lie adjacent (fig. 8).

Insofar as regeneration within a graft usually begins at several loci, later stages show the reconstituted transplant to consist of a number of closely associated units, each bounded by a narrow but well-developed capsule of fibrous connective tissue (fig. 14). The topographic arrangement of the cells is very reminiscent of normal glands (fig. 13). Thus the peripheral cells are irregularly disposed, much as in the zona glomerulosa, though they are generally somewhat larger in size. Most of the graft consists of regular parallel cords of polyhedral cells between which run straight and well-developed capillaries, a picture of the typical zona fasciculata. In some, but not all of the transplants, a loose reticularis arrangement is also found, usually near the center of the glandular mass.

The fasciculate cells after 2 weeks show a large and evenly distributed content of lipoidal droplets (fig. 10), and thus are identical in appearance with corresponding cells of normal glands. The Golgi apparatus forms a fine reticulum between these droplets. On the other hand, the peripheral cells, smaller in size and with few or no lipoidal inclusions, possess a small condensed Golgi net apposed to one side of the nucleus.

Serial sections of 17-day implants and of later stages reveal no recognizable traces of medullary cells. Apparently these have completely degenerated and have been absorbed by connective tissue elements.

A phenomenon, not seen in normal glands, is the occurrence within the engrafted tissue of rounded or irregular spaces often gorged with erythrocytes (fig. 14), but more frequently without cellular content or with very few red cells (fig. 9). These spaces are sometimes lined by endothelial cells, when they appear to be local distentions of capillaries, but usually they are without lining. In the latter case the contained erythrocytes seem to have been extravasated from neighboring vessels. In fact, scattered red blood cells, not enclosed in regular channels, had been noticed among regenerating cells ever since vascularization first set in, and possibly are the result of early hemorrhages of the invading capillaries. At the borders of the distended tissue spaces large globules are prominent, which might be secretions from the adrenal cells. Smaller droplets, similar in appearance, are present in the cytoplasm of these cells.

In all but five of the experimental animals each of the two or three grafts implanted 'took' and was recovered. In the others one of them was resorbed. Grafts growing for 2, 3, or 4 months were usually several times the size of the pieces originally implanted. In two of the forty-six animals hypertrophied accessory tissue was noted at autopsy, and in another three, serial sections of the adrenal sites revealed the presence of microscopic amounts. However, this did not affect the growth of the implants.

Evidences of function. Evidence for the physiological activity of the ultracentrifuged grafts was obtained from several sources.

Cytological indications of function, involving the appearance of secretory droplets in the cytoplasm and correlated changes in cytoplasmic organoids, have already been described.

The survival period of adrenalectomized animals without implants was determined from twenty-six individuals kept under the same conditions as the transplantation group. Death, preceded by typical insufficiency symptoms, occurred in from 4 to 18 days, with 11 days as the average. (Three

additional individuals belonging to this group were killed after 31, 81 and 92 days, respectively, and were seen to possess accessory cortical tissue.) Of the implanted animals in which the grafts were left to grow 20 days or longer, fifteen possessed no accessory cortical tissue. This fact suggests that the implants supplied the cortin necessary for survival.

In eleven animals whose grafts had been growing for 16, 20, 25, 25, 48, 90, 90, 90, 116, 120 and 120 days, respectively, the implants were removed under ether anesthesia, and the subsequent survival period noted. Nine of these animals developed insufficiency symptoms and died in 4, 6, 14, 17, 18, 18, 21, 21 and 23 days. The other two were killed at 34 and 35 days: one possessed a hypertrophied accessory, the other no accessory tissue but one implant which had been unmarked and overlooked at the time of the operation!

Daily weight records also gave striking indication of the efficacy of the grafts. The control animals either lost weight or remained at the same weight level until death. Adrenalectomized rats with implants and no accessories invariably gained in weight after 10 days, and showed normal growth curves. Four of the females bore litters of young 2 months after adrenalectomy and implantation of the centrifuged tissue. The animals which died after surgical removal of the grafts showed daily weight loss following the operation, whereas the two which continued to live for over a month and were shown to possess adrenal tissue, maintained normal weight relations.

DISCUSSION

The effects of centrifugal force on the survival of egg cells have been known for some time. Lyon ('07), stratifying the components of *Arbacia* eggs with a force of 6400 gravity, reports that such treatment does not hinder their subsequent development into normal plutei. He made no close study of the recovery process, but states that displaced pigment very slowly finds its way over the lower hemisphere and finally over the whole egg; the lipoidal cap at the light end of the egg dis-

appears only after a long time if at all. Conklin ('17) carefully investigated the effects of centrifugalization on the eggs of *Crepidula*, using a force of 600 to 2000 gravity. He remarks that "the stratification of egg substances is never complete, but strands of spongioplasm prevent the free movement and stratification of substances according to their relative weight. The spongioplasm is highly elastic and contractile and when it is stretched or distorted it tends to come back to its normal form and to bring back to their normal positions displaced constituents of the cell." More recently Harvey ('33, '34), working with the centrifuge-microscope, makes the striking observation that fertilized sea urchin eggs may cleave even while being rotated at 5000 times gravity. She also finds that streamers of elongated eggs retract very quickly immediately upon removal from the centrifuge, although about an hour is required for a complete return to the original spheroid shape. Beams and King ('36 a), exposing fertilized eggs of *Ascaris* to an ultracentrifugal force of 400,000 gravity for 60 minutes, report that the stratification effects are lost in 12 hours, and that after 48 hours 90% of the eggs have cleaved. Even after 4½ days of continuous centrifuging at 150,000 gravity such eggs were observed to develop normally.

Stratification effects on a wide variety of specialized tissue cells have now been studied by means of the ultracentrifuge, operating at 50,000 to 400,000 times gravity. These include such different types as neurons (Beams and King, '35 a; Brown, '36), spermatocytes (Beams, Gatenby and Muliyl, '36), cells of uterine glands (Beams and King, '34 a), liver (Beams and King, '34 b), thyroid (Hellbaum, '36), submaxillary gland (Lucas, '36), anterior pituitary body (Guyer and Claus, '36 a), adrenal (Dornfeld, '36), cornea (Lucas and Herrmann, '35), embryonic tissues and carcinoma (Beams and King, '36 b; Guyer and Claus, '36 b), and bean root tip (Beams and King, '35 b).

Conclusive evidence that tissue cells subjected to the enormous forces of the ultracentrifuge are not thereby seriously injured or left non-viable, has up till recently been wanting.

Beams and King ('35 a) briefly pointed out that turtle heart centrifuged at 400,000 gravity for $\frac{1}{2}$ hour was still beating, and most workers have emphasized the absence of cytolytic artifacts in carefully fixed preparations. But Guyer and Claus ('36 a and b) were first to show, by transplantation methods, that centrifuged cancer cells, rat embryonic tissue, and anterior pituitary cells are capable of continued growth. The cancer cells, however, were very resistant to stratifying forces, but the embryonic tissue was effectively stratified and showed active subsequent growth. In pituitary cells their results are somewhat comparable to those reported in the present study. Promptly following the cessation of centrifuging, the nucleus returned to the central region of the cell and the various cell granules became redistributed, but the Golgi complex regained its network-like configuration only after 2 or 3 weeks. Both centrifuged and normal transplants disintegrated by the end of 4 weeks. However, since these were homoplastic grafts and the hosts were not hypophysectomized, the death of the transplants is not surprising.

The slow reconstitution of the Golgi apparatus, as reported by Guyer and Claus and by the writer, is very likely not attributable to the immediate effects of centrifuging. It appears, rather, to be associated with secretory activity in the cytoplasm. This is borne out by the fact that in Guyer and Claus' material non-centrifuged transplants showed the same slow return, and that in adrenal transplants, as here described, increase in the quantity of what appear to be secretion droplets accompanies enlargement of the Golgi net. Moreover, in unpublished work on hypophysectomized rats, the writer finds that in the fasciculate zone of the adrenal, retraction of the diffuse Golgi network into a dense juxtannuclear mass occurs concomitantly with the disappearance of such cytoplasmic droplets.

The presence of extravasated blood in the adrenal grafts is much the same as that found by Waterman ('32) in organ primordia transplanted into the omentum of rabbits. The phenomenon is fully discussed in his paper, and no observations were made which might extend what is already known.

The failure of medullary tissue to regenerate is fully consistent with all previous reports on adrenal grafts, though no adequate explanation has yet been put forward. Crowe and Wislocki ('14) regard a 'physiological deficit' as essential for a successful 'take,' but this is certainly not true for a great variety of tissues. However, if valid for the specific instance, it would be of interest to know exactly how such lack of deficit is accounted for. The embryological relation of medullary cells to nervous tissue has also been emphasized, inasmuch as nervous tissue shows little or no regenerative capacity. Jaffe ('27), in making autoplasmic grafts in guinea pigs, was careful to use only cortical fragments, since the presence of medullary tissue inhibited cortical growth. The reverse relation might obtain in other species. No attempt has yet been made to study the effects of transplanting medullary cells only. The stratification effects produced in medullary nuclei by ultracentrifuging are quickly overcome, and in the highly viscous cytoplasm are not marked. In view of this and of the transplantation results with normal tissue, the lack of medullary growth cannot be attributed to the effects of centrifuging.

SUMMARY AND CONCLUSIONS

1. Marked stratification and displacement of cellular components was produced in rat adrenal glands by 30 minutes of ultracentrifuging at a force of 400,000 times gravity.

2. Such glands when subsequently left in isotonic Locke-Lewis solution for 3 hours show an almost complete recovery of the nuclear elements. Lipoidal droplets and Golgi apparatus, however, do not regain their original form and position.

3. Glands centrifuged and halved were autoplasmically grafted into the abdominal muscles. Typical host-transplant reactions are called forth, and vascularization sets in on the third day. With the development of the blood supply, rapid reconstitution and regeneration of cortical adrenal cells occurs, during which the hitherto shrunken Golgi mass expands and small lipoidal droplets appear in the cytoplasm. The fully regenerated grafts show histological features comparable to the cortex of the normal gland, save for the frequent

occurrence of rounded tissue spaces filled with extravasated blood and secretion products.

4. Medullary cells do not regenerate, but are disintegrated completely by the seventeenth day. This phenomenon, however, is not attributable to the effects of centrifuging.

5. That the reconstituted cortical cells possess normal functional capacity is indicated by the appearance in cytological preparations of newly developed secretory droplets and associated changes in organoids; by the fact that implanted animals without accessory tissue outlive adrenalectomized controls; by the fact that surgical removal of the grafts is followed by insufficiency symptoms and death; and by the fact that animals with implants show normal weight gains, whereas completely adrenalectomized ones do not.

6. Evidence from other studies on the survival of centrifuged eggs, and from transplantations of centrifuged somatic tissues shows that the stratification and morphologic displacement of cellular components has no necessarily deleterious effect on these components or on the viability and functional capacity of the cells as a whole. The use of centrifuging methods for the determination of relative specific gravities of cell parts may therefore be considered valid.

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PLATE 1

EXPLANATION OF FIGURES

3 Two-day graft of ultracentrifuged adrenal, showing infiltration of neutrophils. Bouin; iron-hematoxylin. $\times 97$.

4 Seven-day graft of ultracentrifuged adrenal, showing fibroblast invasion. Bouin; iron-hematoxylin. $\times 97$.

5 Ultracentrifuged cortical adrenal cells, after 3 hours in isotonic Locke-Lewis solution. Bouin; iron-hematoxylin. $\times 925$.

6 Ultracentrifuged medullary adrenal cells, after 3 hours in isotonic Locke-Lewis solution. Bouin; iron-hematoxylin. $\times 925$.

7 Fourteen-day graft of ultracentrifuged adrenal, showing reconstitution of cortical cells. Note relation to blood supply and unrecovered cells at left. Bensley; iron-hematoxylin. $\times 330$.

8 Fourteen-day graft of ultracentrifuged adrenal, showing degenerating medullary tissue between regions of reconstituted cortex. Bouin; iron-hematoxylin. $\times 97$.

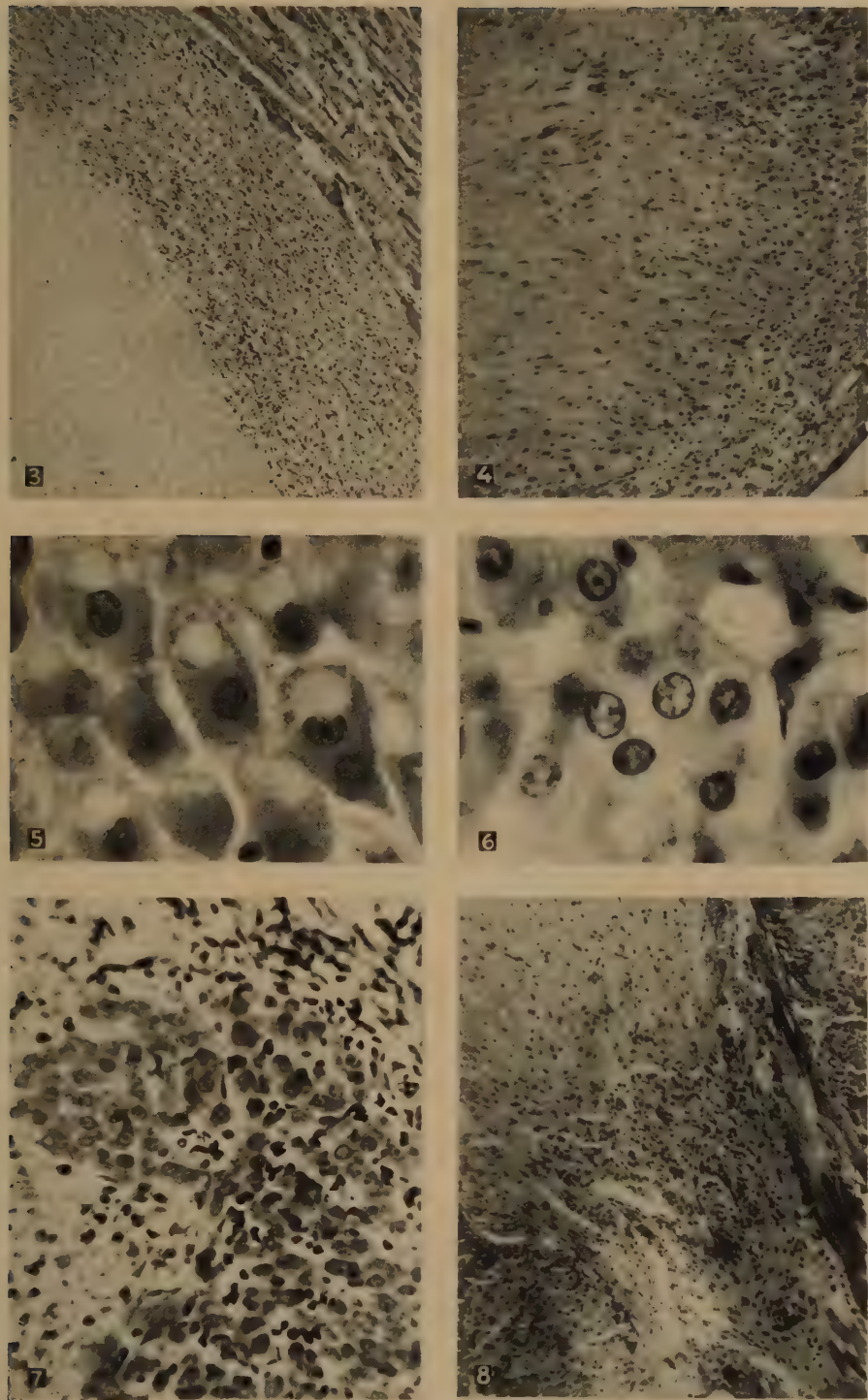


PLATE 2

EXPLANATION OF FIGURES

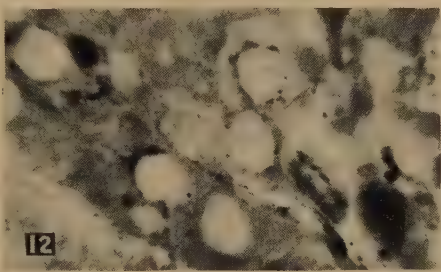
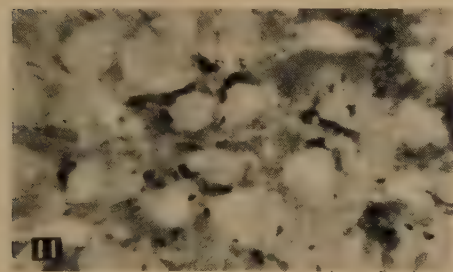
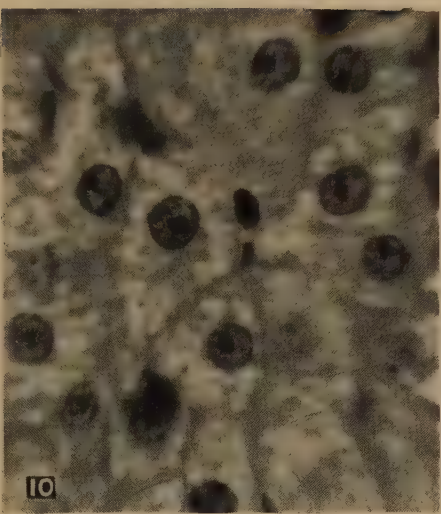
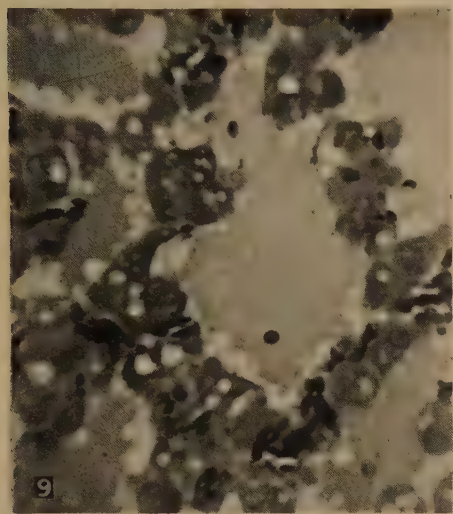
9 Tissue spaces in 90-day graft of ultracentrifuged adrenal. Note extravasated erythrocytes and probable secretion globules bordering cortical cells. Bensley; iron-hematoxylin. $\times 425$.

10 Cortical cells in 60-day graft of ultracentrifuged adrenal. Note normal appearance and presence of evenly distributed secretion droplets. Bouin; iron-hematoxylin. $\times 950$.

11 and 12 Cortical cells in 10-day graft of ultracentrifuged adrenal, showing Golgi apparatus about nuclei. Sevringhaus-Champy; post-osmicated. $\times 950$.

13 Regenerated cortical tissue in 60-day graft of ultracentrifuged adrenal. Note histological arrangement of cells into glomerular and fasciculate zones, and interstitial capillaries. Bouin; iron-hematoxylin. $\times 72$.

14 Fifty-day graft of ultracentrifuged adrenal. Note three regeneration units, central reticulate zone, blood spaces, fibrous capsule, and absence of medulla. Bouin; iron-hematoxylin. $\times 23$.



STUDIES ON THE CREEPER FOWL

XI. CASTRATION AND LENGTH OF BONES OF THE APPENDICULAR SKELETON IN NORMAL AND CREEPER FOWL

WALTER LANDAUER

Storrs Agricultural Experiment Station, Storrs, Connecticut

The data which will be reported here bear on the question whether or not castration of young cockerels has an effect on growth in length of the bones of the extremities.

The evidence available on this problem, as reported in the literature, is conflicting. The conclusions of the earlier reports concerning the effects of castration on bone growth in fowl must be discounted since they were based on entirely inadequate material. Both Sellheim (1899) and Pirsche ('02) had data from only two capons each, and Poncet's ('03) statements merely reiterate the findings of Pirsche. This leaves only the publications of Masui and Hashimoto ('27) and of Hutt ('29). Working with White Leghorns Masui and Hashimoto caponized seventy-one males and used forty-five cockerels for control. Castration was done at three different ages, namely, 25 to 30 days, 50 to 60 days, and at about 70 days. In none of the three groups did these authors find that growth in length of the long bones was in any way altered by castration. Hutt, on the other hand, concluded from his data that capons show changes in length and in proportions of the bones of the extremities. His material consisted of sixteen capons, thirty-six males and fifty-three females belonging to the Leghorn group of breeds, the majority being Brown Leghorns. The birds had been received for autopsy from various sources; all were adult. As far as the capon skeletons are concerned Hutt's conclusions with regard to length and proportions are

as follows. Mean absolute length for all bones of the extremities is greater in capons than in normal males, except length of the distal phalanx of the wing. The differences are statistically significant for humerus, tibiotarsus, and three phalanges of the (third) toe. Hutt suggests that with more ample material the differences in most of the other bones probably would also have been significant. Increase of bone length was more pronounced in the legs than in the wings. In addition to this change in relative proportions it was found in capons that in the wing the distal bones were shortened relative to the humerus, while in the leg the distal bones (except one phalanx) showed an increase in length relative to the femur. Castration, with other words, according to this material brings about gradients of relative increase in length which are opposite in direction in the wings and in the legs.

The purpose of the present study was 1) to ascertain whether caponization leads to increased growth in length of the bones of the extremities, and, if so, to study further its differential effect on the various bones; and 2) to determine if bone growth of Creeper chickens, on account of the chondrodystrophic dwarfism of this breed, responds differently to castration than bone growth of normal male fowl.

MATERIAL AND METHODS

All chicks used in this experiment came from a mating of Creeper pullets and a normal cockerel. Groups of full brothers, Creeper and normal, were used for castration and as controls. Castration was done at once on removal of the chicks from the incubator. All capons and control males were killed when they had reached the age of 10 months. The question whether the effect of castration on bone growth is only one of lengthening the period during which growth takes place, or whether there is also a stimulation of increase in length during the normal growth period, has always remained open. By castrating the chicks at hatching time and by keeping them to at least twice the age during which normally growth in length of the long bones occurs, ample opportunity

was given for the expression of any possible effects of castration. In a few of the operated animals testicular tissue was found on autopsy, and these were rejected. Length of dorsum, i.e., the distance from the first thoracic vertebra to the end of the pygostyl, was measured after removal of the plumage. Subsequently, all the bones of the left wing, and the long bones and phalanges of the third digit of the left leg were dissected out and their lengths measured. In reporting these measurements the means are given with their standard errors.

RESULTS

The data for absolute length of the different bones and for length of the dorsum are reproduced in table 1. It is evident from these figures that neither castrated and control normal males nor castrated and control Creeper males showed significant differences in length of any of the bones of the extremities. Length of the various bones relative to body size, as expressed by indices based on length of dorsum, also do not show significant differences in a single instance (table 2); this holds both for Creeper chickens and their genetically normal sibs. Finally, with regard to the proportions of length between different bones of one and the same extremity or between bones of leg and wing, the figures in table 3 indicate complete absence of significant differences between castrated and control normal males, while among the Creeper capons and control males a difference which is as great as or greater than the threefold standard error exists in only one of the fourteen calculated indices. This exception relates to the femoro-humeral length index, the difference amounting to 2.0 ± 0.61 . This can scarcely be taken as an indication of a real difference.

TABLE 1

Means and standard errors of absolute length of bones of castrated and control fowl

BONE		CASTRATED NORMAL N = 21	CONTROL NORMAL N = 39	CASTRATED CREEPER N = 17	CONTROL CREEPER N = 26
		cm.	cm.	cm.	cm.
Left wing	Humerus	8.10 $\pm$ 0.049	8.10 $\pm$ 0.045	6.79 $\pm$ 0.048	6.66 $\pm$ 0.048
	Ulna	8.12 $\pm$ 0.058	8.09 $\pm$ 0.044	6.40 $\pm$ 0.042	6.31 $\pm$ 0.047
	Radius	7.33 $\pm$ 0.052	7.34 $\pm$ 0.041	5.68 $\pm$ 0.040	5.61 $\pm$ 0.047
	Carpometacarpus	4.38 $\pm$ 0.033	4.36 $\pm$ 0.024	3.60 $\pm$ 0.023	3.53 $\pm$ 0.026
	Digit III, phalanx 1	1.53 $\pm$ 0.013	1.55 $\pm$ 0.013	1.44 $\pm$ 0.019	1.45 $\pm$ 0.013
Left leg	Digit III, phalanx 2	1.53 $\pm$ 0.018	1.49 $\pm$ 0.014	1.40 $\pm$ 0.023	1.38 $\pm$ 0.016
	Femur	8.62 $\pm$ 0.059	8.67 $\pm$ 0.047	7.13 $\pm$ 0.066	7.09 $\pm$ 0.055
	Tibia	12.78 $\pm$ 0.110	12.87 $\pm$ 0.072	8.97 $\pm$ 0.100	9.00 $\pm$ 0.075
	Tarsometatarsus	9.26 $\pm$ 0.091	9.38 $\pm$ 0.065	6.60 $\pm$ 0.055	6.53 $\pm$ 0.058
	Digit III, phalanx 1	2.02 $\pm$ 0.019	2.01 $\pm$ 0.013	1.90 $\pm$ 0.014	1.87 $\pm$ 0.012
	Digit III, phalanx 2	1.62 $\pm$ 0.017	1.63 $\pm$ 0.016	1.55 $\pm$ 0.015	1.51 $\pm$ 0.016
	Digit III, phalanx 3	1.57 $\pm$ 0.016	1.56 $\pm$ 0.011	1.51 $\pm$ 0.013	1.47 $\pm$ 0.014
Dorsum		20.6 $\pm$ 0.24	20.3 $\pm$ 0.15	19.6 $\pm$ 0.18	19.5 $\pm$ 0.13

TABLE 2

*Means and standard errors of relative length of bones of castrated and control fowl
(relative length = absolute length $\times$ 100, divided by length of dorsum)*

BONE		CASTRATED NORMAL N = 21	CONTROL NORMAL N = 39	CASTRATED CREEPER N = 17	CONTROL CREEPER N = 26
		cm.	cm.	cm.	cm.
Left wing	Humerus	39.4 $\pm$ 0.39	40.0 $\pm$ 0.27	34.7 $\pm$ 0.31	34.1 $\pm$ 0.20
	Ulna	39.5 $\pm$ 0.46	39.9 $\pm$ 0.26	32.7 $\pm$ 0.29	32.3 $\pm$ 0.21
	Radius	35.6 $\pm$ 0.38	36.2 $\pm$ 0.24	29.1 $\pm$ 0.26	28.7 $\pm$ 0.18
	Carpometacarpus	21.3 $\pm$ 0.22	21.5 $\pm$ 0.14	18.4 $\pm$ 0.19	18.1 $\pm$ 0.10
	Digit III, phalanx 1	7.45 $\pm$ 0.08	7.62 $\pm$ 0.06	7.36 $\pm$ 0.10	7.46 $\pm$ 0.06
Left leg	Digit III, phalanx 2	7.41 $\pm$ 0.10	7.35 $\pm$ 0.04	7.15 $\pm$ 0.14	7.04 $\pm$ 0.07
	Femur	41.9 $\pm$ 0.38	42.6 $\pm$ 0.30	36.4 $\pm$ 0.33	36.3 $\pm$ 0.21
	Tibia	62.3 $\pm$ 0.61	63.5 $\pm$ 0.41	45.9 $\pm$ 0.62	46.0 $\pm$ 0.33
	Tarsometatarsus	45.0 $\pm$ 0.51	46.3 $\pm$ 0.36	33.8 $\pm$ 0.39	33.4 $\pm$ 0.20
	Digit III, phalanx 1	9.79 $\pm$ 0.10	9.92 $\pm$ 0.07	9.70 $\pm$ 0.11	9.59 $\pm$ 0.06
	Digit III, phalanx 2	7.87 $\pm$ 0.10	8.04 $\pm$ 0.08	7.91 $\pm$ 0.09	7.73 $\pm$ 0.06
	Digit III, phalanx 3	7.63 $\pm$ 0.08	7.69 $\pm$ 0.05	7.75 $\pm$ 0.09	7.51 $\pm$ 0.07
Digit III, phalanx 4		6.61 $\pm$ 0.12	6.70 $\pm$ 0.07	6.60 $\pm$ 0.06	6.36 $\pm$ 0.05

TABLE 3
Means and standard errors of length indices of various bones of castrated and control fowl

INDEX	CASTRATED NORMAL N = 21	CONTROL NORMAL N = 39	CASTRATED CREEPER N = 17	CONTROL CREEPER N = 26
Ulna $\times$ 100	100.3 $\pm$ 0.48	99.9 $\pm$ 0.14	94.3 $\pm$ 0.23	94.7 $\pm$ 0.14
Humerus				
Radius $\times$ 100	90.5 $\pm$ 0.26	90.6 $\pm$ 0.20	83.7 $\pm$ 0.26	84.2 $\pm$ 0.18
Humerus				
Carpometacarpus $\times$ 100	54.1 $\pm$ 0.28	53.8 $\pm$ 0.18	53.0 $\pm$ 0.27	53.0 $\pm$ 0.21
Humerus				
Digit III, phalanx 1 (wing) $\times$ 100	18.9 $\pm$ 0.13	19.1 $\pm$ 0.15	21.2 $\pm$ 0.24	21.9 $\pm$ 0.17
Humerus				
Digit III, phalanx 2 (wing) $\times$ 100	18.8 $\pm$ 0.20	18.4 $\pm$ 0.17	20.6 $\pm$ 0.35	20.6 $\pm$ 0.18
Humerus				
Tibia $\times$ 100	148.0 $\pm$ 0.53	148.0 $\pm$ 0.48	126.0 $\pm$ 0.92	127.0 $\pm$ 0.73
Femur				
Tarsometatarsus $\times$ 100	107.0 $\pm$ 0.65	108.0 $\pm$ 0.48	92.6 $\pm$ 0.60	92.1 $\pm$ 0.45
Femur				
Digit III, phalanx 1 (leg) $\times$ 100	23.4 $\pm$ 0.17	23.2 $\pm$ 0.16	26.6 $\pm$ 0.18	26.4 $\pm$ 0.11
Femur				
Digit III, phalanx 2 (leg) $\times$ 100	18.9 $\pm$ 0.15	18.8 $\pm$ 0.20	21.7 $\pm$ 0.18	21.3 $\pm$ 0.15
Femur				
Digit III, phalanx 3 (leg) $\times$ 100	18.3 $\pm$ 0.17	18.0 $\pm$ 0.09	21.3 $\pm$ 0.21	20.7 $\pm$ 0.11
Femur				
Digit III, phalanx 4 (leg) $\times$ 100	15.8 $\pm$ 0.27	15.7 $\pm$ 0.17	18.1 $\pm$ 0.22	17.5 $\pm$ 0.19
Femur				
Femur $\times$ 100	106.0 $\pm$ 0.38	107.0 $\pm$ 0.22	105.0 $\pm$ 0.51	107.0 $\pm$ 0.33
Humerus				
Tibia $\times$ 100	158.0 $\pm$ 0.64	159.0 $\pm$ 0.49	140.0 $\pm$ 1.16	143.0 $\pm$ 0.92
Ulna				
(Femur + tibia + tarsometatarsus) $\times$ 100	148.8 $\pm$ 0.52	150.4 $\pm$ 0.33	134.7 $\pm$ 0.87	137.5 $\pm$ 0.59
Humerus + ulna + carpometacarpus				

DISCUSSION

Our results indicate complete absence of any effect of castration on growth in length of the bones of the extremities in male fowl. In this our data agree with those obtained by Masui and Hashimoto. Our observations are at variance with those reported by Hutt, and for this discrepancy we are unable to offer an explanation, unless it is to be found in the manner in which Hutt's material had been collected. The conclusion reached by Horowitz ('34), viz., that the only size difference between capons and normal cocks is brought about by greater fat accumulations in the capons, also agrees well with our findings.

The lack of effect of castration on growth in length of the long bones apparently is more general than had been supposed by earlier writers. All the more recent experimentation with rats (Hatai, '15; Lawless, '36), guinea pigs (Moore, '22; Sumulong, '25), and rabbits (Poppe, '32) has given either clearly negative results or differences of doubtful statistical significance. For the large domestic animals, judging from the survey given by Kříženecký ('32), the evidence for an increase of long bone length after castration seems inconclusive. Moore's ('32) statement with reference to body size, namely, that it is yet to be demonstrated whether consistent effects appear in any mammal after removal of the testes, certainly applies to absolute and relative length of the long bones of both birds and mammals. The only exception to this statement at present appears to be man for whom Pittard ('34) recently has given new and convincing evidence for a striking relative increase in length of the long bones following early castration. It remains to be shown whether this response of the human body to castration is related to the longer growth period or whether it is caused by other factors.

SUMMARY

Castration of male chicks at hatching time did not produce any changes in absolute or relative length of the bones of the extremities. This statement applies to both normal and Creeper fowl.

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BOOK REVIEW

THE LUNG. By William Snow Miller, Emeritus Professor of Anatomy in the University of Wisconsin. Charles C. Thomas, Springfield, Illinois, 1937. 209 pp. \$7.50.

This instructive and beautiful monograph is an epitome of the knowledge of pulmonic morphology which this well-known anatomist has brought to light in 47 years of close study. It gives first a brief survey of the gross anatomy of the lungs and then a systematic description of the gross and microscopic structure of the trunk, branches and complex terminals of the bronchial tree, with related blood vessels, lymphatics, lymphoid tissue, nerves and pleura. The salient features are adequately summarized in a brief chapter entitled 'Key Points.' Next there is an historical sketch in which the reader ascends pleasantly the evolutionary steps in our understanding of the lung, as laid down by Malpighi, Reisseisen, Kölliker, Laguesse and others. Miller's conceptions of the pulmonic acinus are synopsized in the final pages. There is a valuable bibliography in which are listed 222 articles, selected from the author's encyclopaedic collection, and ranging in date from the time of Aristotle to the present. The full index is very helpful.

There are no less than 152 excellent illustrations. More than two-thirds are from the author's works, and at least 33 are original for this book. The many colored ones are particularly appealing. Of the figures from other writers most are inserted for their historical value. As well as to the author and publisher, commendation is due to the Collegiate Press of Menasha, Wisconsin, for this attractive feature, and for the general excellence of type work and binding. The style is terse and clear, and serves magically to bring us into the presence of the master himself as he describes and interprets the great accumulation of evidence with which he deals, pointing frequently now to this chart, now to that model, and withal contriving to instill in us that consuming enthusiasm which he must have felt continuously in carrying out his researches.

That Miller is a master craftsman with the Born method of wax plate reconstruction, and its modifications and improvements, is particularly well realized by those who, like the reviewer, have had the rare pleasure of seeing and handling his models, and of having them

demonstrated by their executant. The book is filled with good illustrations of many of these, as of the tracheal and bronchial cartilages, the air spaces, lymphatics, pulmonic blood vessels and the bronchial musculature. Much of the success of this investigator is due to his patience and sureness in the use of this technic. He is equally skilled in graphic methods of reconstruction, and in all histological manoeuvres, including the successful injection of blood vessels with colored masses, the staining of sections in a multitude of ways, and the preparation of those invaluable aids—serial sections. But the fabrication of scientific preparations, important though it undoubtedly is, is valueless without their proper interpretation, and it is in this way that Miller has given to the world much of value. "Wise men," said William Harvey, "must learn anatomy, not from the decrees of philosophers, but from the fabric of Nature herself," and Miller has gone to that fabric in the common mammals as well as in man—for his conceptions of structure.

Throughout the book there is also abundant evidence that the author appreciates the significance of the transitions, so frequently found, between normal and abnormal structures as, for instance in his description of the metamorphosis of simple columnar into stratified squamous epithelium in a bronchus taken from a tuberculous individual in whom constant coughing had subjected the epithelium to perpetual irritation. His discussion of pigment deposits in the lung is another such example.

The sharp visualization of the structural unit, or lobule, of the lung is absolutely essential to the anatomist, for without it he cannot take even the first step toward an understanding of lung morphology. But this has been found difficult of attainment, since these units are so complex and so intricately dovetailed together that their precise definition has resisted the efforts of investigators for many decades. Small wonder, then, that the anatomical world gratefully acknowledges the debt which it owes to Miller for revealing this lobule in such simplified and diagrammatic form that everyone can comprehend it. This he has done by making careful wax plate reconstructions of it from accurate serial sections, by providing good illustrations of it, and by describing it in the helpful words of a man who has a true feeling for form (chap. IV).

This unit, or 'primary lobule,' has as its stem, according to Miller, the alveolar duct of Kölliker, and this, in his conception, opens into three to six globular and sacculated spaces to which he gives the name 'atria.' Each atrium, he says, opens into a variable number of alveolar sacs (two to five in the cat's lung) which are the 'infundibula' of older workers. The position of the atrium, and its intercommunications, are plainly indicated in his figure 28. His

figure 29 shows two atria in a specially made model from a human lung. It is of interest that Miller published his first description of the atrium when he was in Mall's laboratory at Clark University in 1892. Even earlier, in the College of Physicians and Surgeons in the City of New York, 50 years ago, Miller was able to identify as atria the 'spheroidal bodies' which, he tells us, were described by J. West Roosevelt in dissected wax corrosions. Not all workers, it may be admitted, find this section of the airway of sufficient distinctiveness to merit a special name; but anyone who studies Miller's models will be convinced that the space which Miller designates as the atrium is actually there.

Miller takes a conservative position on the problem of the alveolar epithelium, and believes that the alveolus is completely clothed with a continuous layer of nucleated epithelial cells. He feels that he has demonstrated this continuous layer in pathological specimens from cases of pulmonic oedema and pneumonia, where an accumulation of fluid has, in his view, pushed the 'epithelial layer' away from the basement membrane. His figures showing the phenomenon upon which he bases his convictions have already found their way into text books. Many workers, however, now feel that evidence like this from obviously pathological material cannot be taken as the last word in the debate on the alveolar epithelium problem, and they insist that this should be settled from the histological testimony of the normal alveolar wall.

Viewing the alveolar lining as he does, Miller can see alveolar pores only as abnormal openings. "They are," he says, "not to be considered normal structures, but the result of some pathological process which occasions the shedding of the epithelium on diametrically opposite sides of an alveolar wall" (with perforation, presumably, of the ground substance). Not all modern workers subscribe to this interpretation.

With the form of the primary unit established, Miller proceeds to describe and illustrate the structures contributory to it, in their proper relations. The vascular system is, of course, one and inseparable with the lobule. The subdivision of the pulmonary artery lies against the air trunks and spaces of the lobule, and gives off a branch for each atrium, and these, in turn, give off each a branch for each alveolar sac: and he seems to imply that this mode of ramification gives support to his recognition of the atrium as a positive entity. The veins, unlike the arteries, run between the lobules, and arise each from a plexus in the pleura, receiving tributaries from the regions of the atrium, alveolar duct and respiratory bronchiole. In the last-mentioned district we have the interesting transflux by means of capillaries, of the terminal spray of the bronchial artery fountain

into the blood stream of tributaries of the pulmonary venous system. The anatomy of these parts is all well displayed in figure 54. The bronchial blood vessels are fully described and well illustrated with fine colored prints made from specimens in which the veins and arteries were injected with red and blue colored masses, respectively. This is another particularly good section of the book, filled to the brim with information of great value not only to anatomists and physiologists, but to clinicians as well. For instance, he tells us that, "Wounds of the lung which involve the bronchial artery are much more fatal than those which involve the pulmonary artery; this is due to the higher pressure in the bronchial artery, which causes splenization or hemorrhagic infarction extending to the pleural surface, and is almost constantly provocative of empyema. Obstruction of the bronchial artery may occasion infarction and gangrene." Another practical point is that the vasa vasorum of the pulmonary artery are derived from the bronchial arteries.

The lymphatics, of which the lung has an abundant supply, are minutely dealt with in chapter VI. His figure 54 gives a good view of the various plexuses related to the air tubes, pulmonary artery, pulmonary vein and pleura, and of the direction of lymph flow. He stresses, among other points, that no lymphatics are present in the walls of the air spaces distal to the ductuli alveolares, that the vessels around the bronchi and pulmonary artery are intercommunicating, that the lymphatics which accompany the venous radicles which arise from the bronchial tree join the lymphatics of the bronchi, that those lymphatics which accompany the venous radicles which arise from the pleura join the plexus of lymphatics in the pleura, and that there are valves in these latter connections, admitting of flow only toward the pleura. Though he says (pp. 91, 92) that "The pleural lymphatics unite and form a variable number of trunks which drain into the lymph nodes at the hilum," it is not clear just where these are situated. Miller admits (p. 93) that there has been "much confusion in regard to their relationship to the deep lymphatics of the lung." He implies (p. 110) that the pleural lymphatics receive the lymph flow from a layer of lung substance from 2 to 3 mm. in thickness, because in this layer the valves of the lymphatics of the septa between the secondary lobules point toward the pleura, and says that this arrangement of the valves here precludes the flow in the pleural lymphatics from entering the lung. The lymphatics of the pleura itself are, he says, provided with valves that 'open in all directions,' allowing free circulation of lymph within the limits of the pleura. He then repeats his former statement (p. 111) that, "The pleural lymphatics eventually unite to form main trunks which, together with the lymphatics coming from the interior of the lung along the pulmonary

veins, enter the tracheobronchial lymph nodes." By remote implication perhaps we may look to the interlobular septa for these trunks which drain lymph from the pleural lymphatics to those in the interior of the lung. We feel that Miller's very large circle of readers would like more information on this item.

The distribution of lymphoid tissue in the lungs is described in chapter VII, as it is related to the bronchi, the blood and lymph vessels, and the pleura, and there is included a discussion of pigimentary deposits, in which it is brought out that the presence of irritating particles of carbon leads to an increased amount of lymphoid tissue. The nerves are presented in chapter VIII, in which acknowledgment is made of the included work of Prof. O. Larsell, who at one time acted as Miller's assistant at the University of Wisconsin.

In considering the pleura, Miller distinguishes between those of the thin and thick varieties, and points out that to those of the thick type, as in man, the bronchial artery supplies blood. The tissue content is well analyzed.

Among the useful 'Key Points' we note that it is in the 'transitional zone' between the bronchial arterial and pulmonary circulations that tubercles are most apt to develop, and he comments that, "Possibly a retardation of the circulatory force at this point may be a determining factor."

All of this treatment of the lobule and its associated vessels and other parts is admirably ushered in at the beginning of the book by a description of the lungs as a whole and of the trunk and main branches of the airway; and in this connection we would like to express our admiration of the way Miller has handled the presentation of the bronchial musculature, on which he has done an enormous amount of work. His figures are very illuminating. This 'muscle of Reisseisen' he describes as being arranged in a 'geodesic network'—a very appropriate term. In the lobule he points out that the muscle belongs to the air tube and not to the alveolus. The description of the tracheal and bronchial cartilages is very useful.

But space will not permit of more than mention of a few of the good points in this admirable volume. That it has had an enthusiastic reception is proclaimed by the exhaustion of the first edition. It is no secret that men have been glad to pay a high premium to possess a copy. The author, with indefatigable industry, although now at the age when most men are glad to lay down their labors, is actively engaged in the writing of the second edition, and we feel sure that this will be welcomed just as cordially as was the first.

C. C. MACKLIN

NEW BOOKS AND PUBLICATIONS

AIDS TO PHYSIOLOGY, by Henry Dryerre. 1937. Size, $4\frac{1}{4} \times 6$ inches. Pages vii + 295. 63 illustrations. Index. Cloth. Price, \$1.25. William Wood and Company, Baltimore.

As a condensed summary presenting salient facts of physiology in a concise and logical order, this small book can give great assistance to the student in collecting and organizing information derived from larger textbooks and lecture notes. The new edition has been largely rearranged and rewritten, new illustrations have been introduced, and a new chapter on reproduction added.

TRANSACTIONS OF THE WESTERN SURGICAL ASSOCIATION, 46th annual meeting, Kansas City, Missouri, December 11 and 12, 1936. 1937. Size, 6×9 inches, 482 pages. Illustrated. Index. Cloth. Bruce Publishing Company, St. Paul and Minneapolis.

The volume contains lists of members, officers, previous meetings, and an account of the forty-sixth meeting. It gives in full the text of twenty-three papers and addresses. The articles and discussions cover a wide field, including the use of morphine-scopolamine anesthesia, the control of constipation by sympathectomy, transplantation of tendons and fascia, preventive surgery, and many other problems of interest to the surgeon.

THYROID STIMULATING AND GONADOTROPIC HORMONES OF THE HUMAN ANTERIOR PITUITARY GLAND AT DIFFERENT AGES AND IN PREGNANT AND LACTATING WOMEN ¹

JOHN SAXTON AND LEO LOEB

*Department of Pathology, Washington University School of Medicine,
St. Louis*

In the former investigations Kunkel and Loeb ('35) tested the effect of human anterior pituitary glands on thyroid and ovaries as well as on secondary sex organs in the sexually immature female guinea pig. They noted the presence of thyroid stimulating hormone. In the ovaries, processes both of growth maturation and granulosa and theca luteinization were found in various combinations, but pure maturation effects in the granulosa of large follicles, similar to those characteristic of the guinea pig anterior pituitary, were not observed. In the vagina partial proliferation, as a rule, took place. In the uterus in many cases there were changes in the mucosa which indicated a stimulation of the connective tissue cells, and which led in some instances to a predecidual condition; in the mammary gland a restricted proliferation, sometimes associated with moderate signs of secretion in acinus cells, was seen. In our continued experiments we extended these investigations by studying the effects of age, pregnancy and lactation on the hormone content of the human anterior pituitary gland.

¹We are indebted to Dr. Newton G. Evans, pathologist to the Los Angeles County General Hospital, for a number of the human anterior pituitary glands used in these investigations. These investigations were carried out in part with the aid of a grant from the International Cancer Research Foundation.

In recent years some observations on variations in the action of human anterior pituitary glands have been made by Philipp ('30) and by Wirz ('23-'33). They implanted the gland tissue into mice and used the criteria introduced by Zondek as tests for the kind of hormones which occur in the hypophysis. The development of mature follicles and blood points indicated the presence of follicle stimulating hormone and the formation of corpora lutea signified the presence of lutein hormone. These ovarian changes could be detected by the naked eye. Philipp transplanted the hypophyses of four pregnant women who died shortly before the end of pregnancy; in one case all the mice with implants died prematurely and in the other three cases he found no changes in the ovaries of the mice. After implantation of the hypophyses from two non-pregnant women past the stage of sexual activity, and also from a young woman who had been castrated by radium, he did not find maturation or ovulation in the ovaries of the mice, but noted hemorrhages into follicles and corpora lutea atretica. No definite conclusions were drawn. Wirz implanted pieces of hypophyses without separating anterior and posterior lobes. The tissue was immersed in ether for 24 hours previous to implantation. In the hypophyses from fetuses, infants and children up to 5 years of age, this investigator could demonstrate only Prolan A and Prolan B appeared in the pituitary only a short time before puberty. Prolan B is therefore produced in appreciable amounts only during the time of puberty and afterward, and Wirz concluded that the resting state of the ovaries in young children is caused mostly by the condition of the pituitary, and much less by that of the ovary. In the hypophyses of postpartum cases, examined in the first days after delivery, no sex hormone could be demonstrated. If pregnancy was interrupted in the early months, prolane reappeared at an earlier date than if pregnancy had progressed farther. After the normal ending of pregnancy Prolan A was demonstrable only after about 2 to 3 weeks and Prolan B after 4 to 5 weeks. At the time when Prolan B could be found, the first ovulation usually occurs.

These findings seemed to explain the fact that ovarian function begins 4 weeks after delivery and that at 6 weeks the first menstruation appears. The amenorrhoea in the first 6 weeks after delivery seems therefore to depend upon lack of activity of the anterior pituitary lobe, while the subsequent amenorrhoea during the period of lactation is dependent upon the ovary.

In our investigations we used the guinea pig, instead of the mouse, as the test animal; moreover, in every case the effect of the implantation of the anterior pituitary gland was studied microscopically in serial sections of the ovary; in the majority of cases also the secondary sex organs were examined by means of stained sections. In this way we obtained a more adequate picture of the changes produced in the ovaries and secondary sex organs by the anterior pituitary hormones. Furthermore, we compared in every instance the effects of these implants on the ovaries with the effect exerted on the thyroid glands. Thus we were able to determine whether any relations existed between the gonadotropic hormones and the thyroid stimulating hormone.

In the majority of cases we implanted one-fourth of a human anterior pituitary gland, obtained at autopsy, each day on four successive days into pockets in the subcutaneous tissue; the pocket was then closed with two sutures. In some cases only a part of a gland instead of a whole gland was implanted. Female guinea pigs, in most instances with an initial weight of 180 to 190 gm., were used for these experiments; but the initial weight of some of these animals was as low as 170 gm. and that of others as high as 200 gm. Examination took place on the fifth day following the first implantation.

I. The action of anterior pituitary glands of children

The anterior pituitary glands of fourteen children were studied. We can arrange them in three groups.

a. Group I consisted of the glands of two male children, one 7 and the second one 14 months old. These anterior pituitaries did not produce any changes, neither maturation nor

luteinization, in the ovaries of the guinea pigs. The ovaries showed the typical follicles found in guinea pigs between the ages of 185 to 200 gm. The vagina was resting; in the uterus some mitoses were seen in the uterine glands and a restricted number of mitoses was observed in the connective tissue of the mucosa. In the mammary gland signs of some secretion and slight proliferation were noted. The lack of any effect in these experiments was probably due to the small size of the glands in such young children, the amount of hormones thus introduced remaining below the threshold of activity. The thyroid glands of the guinea pigs showed in one case a slight and in the second case a very slight hypertrophy.

b. Group II consisted of eight anterior pituitary glands, four from male and four from female children. The male children were $3\frac{1}{2}$, 5, 8 and 11 years old; the female children were 7, $8\frac{1}{2}$, $9\frac{1}{2}$ and $9\frac{1}{2}$ years. The size of these anterior pituitary glands varied between about one-half and three-fourths of the gland of an adult. In two male children, $3\frac{1}{2}$ and 8 years old, the anterior pituitary was slightly less than one-half the adult size. In all guinea pigs with implants of these glands maturation of some of the follicles took place, the size the mature follicles reached varying in different cases; in some of the experiments the mature follicles were very large, while in others they did not reach full size. Follicular granulosa degeneration was diminished in the majority of the ovaries. Luteinization processes, including formation of interstitial gland, were entirely lacking. In those cases in which the sections of the vagina were examined squamous epithelium with or without keratinization was found; only in one case the formation of squamous epithelium was incomplete. The uterus showed more or less an oestrous state. In the mammary gland proliferation of different degrees was noted. We may then conclude that the maturation inducing hormones is present in the anterior pituitaries of children and that the oestrin given off by the ovaries induces growth processes in the secondary sex organs.

c. Group III consisted of four anterior pituitary glands. In three of these cases the glands were from female children, 2, 8 and $9\frac{1}{2}$ years old. In one case the gland was that of a male child 12 years old. The anterior pituitary gland from the 2-year-old girl was only from one-fourth to one-third the size of an adult gland. There were found in the ovary of the guinea pig, in which was implanted this anterior pituitary, fairly large follicles with net-granulosa, indicating a condition of the follicle often preceding maturation. There was occasionally observed a luteinization of the theca interna. Some pseudolutein bodies were seen, apparently resulting from an ingrowth of connective tissue and vessels into the granulosa of large follicles which luteinized during this process. In some follicles there was a moderate increase in the size of the theca interna and perhaps also a slight increase in the size of the granulosa; when connective tissue grew into the granulosa of such follicles a rudimentary type of luteinizing connective tissue atresia took place. There was a moderate formation of interstitial gland in the medulla. The vagina was in a resting state and in the connective tissue of the uterine mucosa a slight mitotic stimulation was noticeable. Notwithstanding the small size of the anterior pituitary gland in this case, it exerted some luteinizing effects on theca interna structures and also on the granulosa. In the second case, that of an 8-year-old girl, there was distinct growth and maturation of follicles; granulosa degeneration was lacking, but some premature maturation of follicles was observed. There was no interstitial gland in the medulla, but there was a slight luteinization of theca interna around follicles during the stage of late connective tissue atresia. The vagina showed slight proliferation, the uterus approached oestrus and the mammary gland was proliferating. In the third case, that of a $9\frac{1}{2}$ -year-old girl, we found marked growth of follicles and maturation of granulosa in large follicles; also some premature maturation of the granulosa took place. There was no interstitial gland in the medulla, but in a few follicles in medium to late connective tissue atresia lutein

rings, originating in remnants of granulosa, were found. In the vagina there was formation of squamous epithelium, covered by vacuolar cylindrical epithelium instead of by keratin. The uterus approached an oestrus condition and the mammary gland showed some secretion and proliferation. In the last case, that of a 12-year-old boy, we found pseudo-lutein bodies and lutein rings, which latter developed in follicles during the stage of connective tissue atresia, a moderate formation of interstitial gland tissue in the medulla in addition to rudimentary interstitial gland bodies. The follicles did not reach full size. It is possible we have to deal in this child with a state approaching puberty.

In addition to these cases, the pieces from the anterior pituitary gland of a 13-year-old boy were implanted into a guinea pig at the same time instead of on four successive days. Examination took place on the fifth day. This anterior pituitary was found entirely inactive. No indication of ovarian or thyroid effects was found. The cause for this unusual result is not clear.

Conclusions. In all the anterior pituitary glands of sexually immature children—except the youngest two in which the amount of tissue was too small to be effective—the maturation hormone was found. There may occur in addition in a minority of cases in children, substances in the anterior pituitary gland which induce slight luteinization processes in the granulosa as well as in the theca interna, and which cause a more or less marked inhibition of the growth of the vaginal epithelium. Growth and maturation of follicles in these cases may be complete or incomplete. The thyroid gland showed hypertrophy in all instances; it was however only slight after implantation of the anterior pituitary glands of the two youngest children. Presumably the small size of the anterior pituitary glands was responsible for the partial lack of efficiency in these instances. In six cases, those of children 2, 5, 8, $9\frac{1}{2}$, 11 and 12 years old, the hypertrophy of the thyroid gland was marked. In five cases of children $3\frac{1}{2}$, 7, $8\frac{1}{2}$, $9\frac{1}{2}$, and $9\frac{1}{2}$ years old, there was distinct but moderate

hypertrophy. We see then that the thyroid stimulating hormone was present in the anterior pituitary gland of all children tested, and that it may be associated solely with the growth-maturation hormone. However, an objection might be raised to this conclusion. It might be said that, in analyzing the production of hormones by the anterior pituitary gland by means of implantation of the gland into animals, it is merely the hormone content of the tissue at one particular time which is measured; we determine therefore a static instead of a dynamic condition. Two glands might contain the same amount of maturation hormone, but in one case it might be retained for a much shorter time in the tissue in which it is produced than in the other gland, a more rapid discharge of the hormone being compensated by its more rapid new formation. If a rapid discharge of hormones would take place from the anterior pituitary glands of children, the activity in ovaries and secondary sex organs of female children should be very great, but this is not the case. This interpretation can therefore be excluded and the same conclusion applies also to the states in which the anterior pituitaries were studied by us.

There was a possibility that the dominance of the growth-maturation stimulating factors over those that induce luteinization processes in the anterior pituitary glands of children was due to the relatively small size of these glands in young children which gave the maturation hormone a better chance to assert itself and that it did not prove an actual lack of this hormone.

II. Implantation of fractions of anterior pituitary glands

In order to test this point we made, therefore, five experiments in which we divided the anterior pituitary glands of adult persons into two parts each. In one case the gland from a 63-year-old man was divided into one-fourth and three-fourth parts; in a second case the gland from a 35-year-old man was divided into one-third and two-thirds of an anterior pituitary. In the other three cases, those of a 20-, 40- and

60-year-old man respectively, the glands were divided approximately into halves. Otherwise the method used in implantation and the time of examination were the same as in the previous experiments.

In every case luteinization processes were found in the ovaries, such as the presence of luteinizing connective tissue atresia, the formation of interstitial gland bodies and of medullary interstitial gland tissue. In some instances also pseudolutein bodies and pseudocorpora lutea were noted, and in addition usually a premature maturation of granulosa. A growth of some follicles to a large size and their subsequent maturation was not observed in every case, some variations existing in this respect in the effects exerted by different glands. When the smallest amounts, namely one-fourth and one-third of a gland, were implanted, the luteinization processes were also present, but were perhaps less marked than after implantation of larger quantities. The formation of interstitial gland tissue in the medulla and of interstitial gland bodies was more marked with three-fourths and two-thirds than with one-fourth and one-third of a gland. Also growth of follicles and maturation of granulosa in large follicles were more advanced after implantation of the larger amounts of anterior pituitary gland. A greater weight of the guinea pigs with implants, as well as a gain in weight during the period of transplantation, also seemed to favor a more marked development and subsequent maturation of large follicles. While the relative proportion of growth-maturation and of luteinization processes in the ovaries varied in different cases, both types of conditions were present in every case.

The changes that took place in vagina, uterus and mammary gland corresponded to the conditions found in the ovaries. The vagina almost always showed moderate proliferation, and to an increase in the size of the maturing and mature follicles there corresponded also a stronger proliferation of the epithelium in the vagina. The mammary gland underwent noticeable proliferation in those animals in which

fully mature, large follicles were found in the ovaries. The presence of pseudocorpora lutea, or in some cases perhaps merely the presence of theca interna luteinization, was associated with predecidual changes in the uterus. The thyroid gland showed hypertrophy in every instance, except in the animal which received only one-fourth of an anterior pituitary gland. That this negative result was not due to lack of thyroid stimulating hormone in the anterior pituitary gland, in this particular case, is proven by the fact that in the guinea pig which had received the remaining three-fourths of this anterior pituitary gland, the thyroid hypertrophy was fairly marked. In a second case there was a similar difference in the degree of thyroid hypertrophy between the two animals, one of which received one-third and the second one two-thirds of an anterior pituitary gland respectively. In the former the hypertrophy was very moderate, while in the latter it was very marked. It is also of interest to note that the presence of larger mature follicles may be associated with marked hypertrophy of the thyroid gland.

Conclusions. We may then conclude that the relative lack of luteinization processes in the ovaries after implantation of anterior pituitaries from children is not due to the diminution in size of the gland, but is characteristic of sexual immaturity. We may furthermore conclude that the immature stage of the ovaries and the secondary sex organs in children before the age of puberty is only in part due to the condition of the anterior pituitaries, in which the factors leading to luteinization processes in the ovary are only very imperfectly or not at all developed. The state of the anterior pituitary glands should at least permit the formation of mature follicles in the ovary and consequently oestrous changes in vagina, uterus and mammary gland. The fact that neither formation of mature follicles nor oestrous changes occur in the secondary sex organs indicates that some other condition, perhaps a lack of responsiveness of the ovarian follicles, or some unknown factor which interferes with the action of the anterior pituitary hormone on the ovary, must be active in children.

III. Implantation of anterior pituitary glands from older persons

Nine anterior pituitary glands from men 60 years old or older, and four from women past the menopause were implanted into guinea pigs. The ages of the men at the time of death were: 60, 60, 63, 67, 75, 77, 81, 81 and 90 years; the ages of the women were: 53, 56, 70 and 75 years. Two anterior pituitaries, those from men 60 and 63 years old, were divided into fractions, the fractions representing one-fourth and three-fourths of the gland in one case, and one-third and two-thirds in the other case; each fraction was cut into four equal parts and one piece implanted daily into four guinea pigs. Altogether into fifteen guinea pigs were therefore implanted pieces of anterior pituitary glands obtained from older persons, some of which had died as the result of accident, while the majority had died from various diseases. In the larger number of the guinea pigs the weights underwent no change, or only a very slight gain occurred during the experiment; only in six animals was the gain greater than 5 gm.

In general, the effects of the anterior glands of old persons were about the same as those observed by Kunkel and Loeb after implantation of anterior pituitary glands from young or middle-aged adult persons. The glands from older individuals induced both maturation and various luteinization processes, but the maturation processes were not quite so pronounced as they often were after implantation of rat, rabbit and guinea pig anterior pituitaries. While we found fairly good-sized mature follicles, in no case did we find the very large, fully mature follicles entirely free from ingrowth of connective tissue into the mature granulosa. In some instances we found large follicles with net granulosa and beginning maturation; in the ovaries of other guinea pigs the maturation processes were further advanced in the granulosa, but usually connective tissue and blood vessels began soon to grow into the granulosa, so that early stages of luteinization developed. In other cases maturation, or perhaps early luteinization began prematurely in the granulosa of follicles which had not yet reached

a large size. In general, luteinization processes, in which granulosa as well as theca interna took part, predominated. Luteinizing connective tissue atresia as well as formation of pseudolutein bodies and pseudocorpora lutea were found quite commonly, although especially the latter structures were not found in every animal. In most cases there was a production of interstitial gland bodies and of well-formed interstitial gland in the medulla of the ovary. As so often occurs when maturation and luteinization affect the granulosa of many, including medium-sized or even smaller, follicles, a marked reduction took place in the amount of degeneration of the granulosa which normally occurs in many follicles.

The epithelium of the vagina showed in no case a complete proliferation leading to the production of keratin. However, in one guinea pig, in which many large follicles with net granulosa and many pseudolutein bodies were present, a layer of typical squamous epithelium had developed, but even in this instance a layer of vacuolated, cylindrical epithelium took the place of keratin. In all other animals the proliferation of the vaginal epithelium was only partial, varying in degree in different cases. In the uterus, as a rule, a slight proliferation in the connective tissue of the mucosa was observed, but in some instances it progressed almost to a predecidual state. The mammary gland showed a more marked proliferation only in those cases in which also the epithelium of the vagina had manifested the greatest degree of proliferation; usually a combination of slight proliferation and secretion processes was noted, or in still other instances proliferation was lacking altogether.

We find, therefore, in these ovaries not the fullest development of growth-maturation processes in the follicles, but only an approach to this condition and a relative preponderance of luteinization processes. However, that the growth-maturation producing hormone may be present in sufficient amounts in the anterior pituitary glands of old persons, even if it should not be fully effective, is indicated by an experiment, described by us elsewhere, in which four pieces of the anterior pituitary

gland from an old person were implanted into a second guinea pig after they had been left in a first guinea pig for 4 days. In experiments of this kind the luteinizing factor is partially extracted in the first animal and in the second the maturation processes have therefore a better chance to become more fully manifest.

Conclusions. There is no essential difference between the anterior pituitary glands of old persons, including women past the menopause and men between the ages of 60 and 90 years, and women and men during the sexually active period of life; however, it is possible, although not certain, that on the average a slight diminution in the hormone which causes full maturation of large follicles may be found in the former. The experiment with serial implantation of the anterior pituitary gland, which we have cited, makes it, however, more probable that it is merely the relative preponderance of the luteinizing factors in the human anterior pituitary glands which prevents the maturation effects from appearing with full strength after implantation of the anterior pituitary glands of older persons. Furthermore, there is no noticeable difference between the anterior pituitary glands of the two sexes in older individuals. If then the activity ceases in the ovaries with the menopause, this effect must be due to changes which have taken place in the recipient tissues, the ovaries, rather than in the hormone producing organ, the anterior pituitary glands. It is of course also conceivable that certain processes of a secondary nature interfere at this age with the action of the hormones on the ovaries.

The thyroid stimulating hormone of the anterior pituitary gland of old persons. The thyroid gland of the implanted guinea pigs showed in the majority of cases a moderate hypertrophy. However, in the guinea pigs implanted with the anterior pituitary glands of a 75-year-old woman and of 60, 67, 77 and 90-year-old men the thyroid hypertrophy was more marked. As stated above, in one case in which only one-fourth of an anterior pituitary gland was implanted in a guinea pig, the amount of hormone producing tissue was too small to call

forth definite hypertrophy. There was no correlation noticeable between the degree of hypertrophy occurring in the thyroid gland and the relative preponderance of maturation or luteinization processes in the ovaries. We may then conclude that there is no noticeable diminution in the amount of thyroid stimulating hormone of the anterior pituitary gland during old age.

IV. Implantation of anterior pituitary glands from pregnant and lactating women

We studied the effects of the implantation of the anterior pituitary glands obtained from women at various stages of pregnancy.

Two months after beginning of pregnancy. Implantation of the anterior pituitary gland produced maturation of very large follicles in the ovaries of the implanted guinea pig and in addition a premature maturation of the granulosa of some medium-sized follicles. Connective tissue began to grow into the granulosa of some large mature follicles. In some follicles the granulosa underwent rarification processes so that net-granulosa follicles appeared. Luteinization of theca interna was found in follicles during the stage of late connective tissue atresia, as well as at earlier stages of connective tissue atresia in large follicles. Interstitial gland bodies of considerable size were observed and also areas of interstitial gland in the medulla. In the vagina the squamous epithelium was covered by cylindrical, vacuolar epithelium; the uterus approached the oestrous state. In the mammary gland some proliferation was noted and in the thyroid there was marked hypertrophy. We may therefore conclude that at this early stage of pregnancy maturation as well as theca luteinization producing and thyroid stimulating hormones are present in the anterior pituitary gland.

Four to 6 months after beginning of pregnancy. Implantation of the anterior pituitary glands did not produce any changes in the ovaries, in which were found merely the various kinds of follicles which are seen in normal ovaries. There

was no sign of maturation or luteinization processes. Vagina and uterus were examined only in the first of these two cases; both organs were in a resting condition. In the mammary gland a few mitoses were seen. In contrast with the lack of gonadotropic hormones, the thyroid stimulating hormone was quite active in both guinea pigs, the thyroids having undergone marked hypertrophy.

Eight months after beginning of pregnancy. This was a case of placenta praevia and the patient died 2 hours after delivery. Large follicles and growing follicles of various sizes, as well as follicles in various stages of granulosa degeneration and connective tissue atresia, were found. In addition there was one very large follicle which was not yet mature, but in which the theca interna was hyperemic and slight granulosa degeneration had set in. Although neither full maturation nor luteinization processes were seen, active proliferation of the epithelium of the vagina had led to the production of squamous epithelium which was covered by vacuolated, cylindrical epithelium instead of by keratin. Also the uterus was in an oestrous condition; the mammary gland showed only slight proliferation and some secretion. The thyroid gland showed marked hypertrophy. While in this case no actual maturation of follicles had taken place, there was evidently a noticeable production of oestrin in the very large follicle which showed signs of beginning maturation.

We may include in this series also the anterior pituitary gland of a woman who died 2 hours following spontaneous labor. In this case also maturation and luteinization changes were lacking in the ovary of the implanted guinea pig. The secondary sex organs were in a resting state. In the thyroid gland moderate hypertrophy was found.

Conclusions. We may then conclude that during the first 2 months of pregnancy the anterior pituitary contains both follicular maturation producing and luteinizing hormones, but that following this period neither of these hormones can be demonstrated in the anterior pituitary gland. However, in one case the beginning of maturation of a follicle with production of oestrin was observed in the ovary of a guinea pig.

This lack of hormones in the anterior pituitary of pregnant woman after 2 months is in agreement with the failure of full follicular growth and maturation during this period.

In contrast to the lack of gonadotropic hormones, the thyroid stimulating hormone was present in normal amounts in the anterior pituitary glands during pregnancy. Whether the production of the gonadotropic hormones actually ceases during pregnancy or whether they are inactivated in some unknown way, cannot be determined at present. While in pregnant women maturation of follicles ceases during pregnancy, in the guinea pig it continues; it would therefore be interesting to determine whether the guinea pig anterior pituitary gland also is devoid of the maturation hormone.

The anterior pituitary gland during lactation. We studied the effects of four anterior pituitary glands from lactating women on the thyroid gland, but only in three of these were we able to examine the sex organs. 1) Ten days postpartum we found growing and many mature follicles in the ovaries. Granulosa degeneration was lacking; there was no premature maturation. Vagina, uterus and mammary gland were in an oestrous condition. The thyroid gland was markedly hypertrophic. 2) Thirteen days postpartum we examined only the thyroid gland of the implanted guinea pig; it showed fairly marked hypertrophy. 3) The effects on the ovaries and thyroid gland exerted by the anterior pituitary from a woman 19 days postpartum, were similar to those of the 10-day specimen. However, in a gland removed 20 days postpartum there were present in addition to growing follicles of various sizes, large follicles with net-granulosa and many large, fully mature follicles, a pseudolutein body in transition to a pseudocorpus luteum and a pseudocorpus luteum of not quite medium size. There was a diminution in granulosa degeneration of follicles. Interstitial gland in medulla and other kinds of luteinization processes were not observed. Uterus and mammary gland were in an oestrous condition, but the epithelium of the vagina showed only slight proliferation, presumably due to the inhibiting action of the pseudocorpora lutea. There was marked thyroid hypertrophy.

Conclusions. We see then that as early as 10 days following labor the follicular maturation hormone has again become active, and at 20 days in addition a formation of pseudocorpora lutea has occurred, but other luteinization processes are as yet lacking. The first luteinization processes leading to the formation of lutein bodies and pseudocorpora lutea appear therefore at a time when the second luteinization processes affecting mainly the theca interna have not yet developed. Maturation and luteinization processes of type I appeared thus in our experiments at an earlier date than in the experiments of Wirz. This difference may, perhaps, be due to the fact that we used a more delicate test object, namely the guinea pig ovary, in preference to the mouse ovary which was used by Wirz.

In addition to the anterior pituitary glands from pregnant and lactating women, we had a chance to examine the anterior pituitary gland from a castrated woman who died as the result of a streetcar accident. The ovaries of the guinea pig implanted with this gland showed growing follicles of various sizes and many large net-granulosa follicles; there was one large atypical mature follicle in addition to smaller follicles with maturing or luteinizing granulosa. A transitional stage between a pseudolutein body and pseudocorpus luteum formation was also observed. Many follicles were in luteinizing connective tissue atresia and in the medulla there was some interstitial gland. The vagina showed only slight proliferation, but the uterus was almost predecidual and in the mammary gland there were marked secretions and slight proliferative processes noticeable. The thyroid gland showed moderate hypertrophy.

The anterior pituitary gland from the ovariectomized woman exerted therefore similar effects to those exerted by the glands from normal adults; in both cases we find a combination of follicular maturation and of luteinization processes, and the luteinization processes seem largely to influence the state of the secondary sex organs.

SUMMARY AND CONCLUSIONS

These investigations show that the amount of thyroid stimulating hormone present in the human anterior pituitary gland is, on the whole, constant throughout the various conditions in the human life. In contrast with this constancy of the thyroid-stimulating hormone, the gonadotropic hormones undergo marked changes. During childhood the maturation hormone is found in the anterior pituitary gland, whereas luteinizing effects on the guinea pig ovary are exerted by the anterior pituitary gland not at all, or only in a rudimentary form. During adult life both follicular maturation and granulosa and theca interna luteinization are induced by the anterior pituitary glands. The same applies in old age, in men as well as in women past the menopause. In neither children nor old individuals can the inactivity of the sexual organs be explained altogether by the condition of the anterior pituitary gland. There must be in addition either a lack of responsiveness on the part of the ovary, or there must be a secondary process which neutralizes the effect of the anterior pituitary hormones on the sex organs. During pregnancy following the first few months all gonadotropic activity of the anterior pituitary is lacking, a condition which corresponds to the failure of follicles to mature in the ovaries of pregnant women. It would be of interest to compare in this respect with the anterior pituitaries of pregnant women, those of pregnant guinea pigs in the ovaries of which follicular maturation processes take place actively during pregnancy. Early during the period of lactation, first the maturation effects and soon afterward also the luteinization processes are restored.

On the whole our observations concerning the effects of the human anterior pituitary gland during various phases of life on the ovary of the guinea pig agree with those of Wirz on the ovary of the mouse, although there are certain differences in our findings and interpretations. Furthermore, the finer differentiation between various types of maturation and luteinization processes which the use of the guinea pig ovary makes possible, show that the first type of luteinization, consisting in the formation of pseudolutein bodies and of

pseudocorpora lutea, may occur earlier than the second type of luteinization processes in which the theca interna participates more closely, but in which also granulosa cells may be concerned to some extent; a partial premature maturation of granulosa cells in smaller follicles is associated with this second type of luteinization in many cases. We have furthermore studied in a considerable number of these experiments the changes which took place in the secondary sex organs and found, in confirmation of our previous observations, that a certain degree of proliferation in the epithelium of the vagina may occur without the presence of mature follicles, and furthermore, that the presence of luteinization in the ovaries may inhibit the full growth of the epithelium in the vagina.

There are indications that during these various phases in human life two kinds of antagonisms are exerted in the sphere of the sex organs: 1) Luteinizing processes, such as premature maturation of granulosa and of theca interna, luteinizing connective tissue atresia, early formation of pseudocorpora lutea of various sizes, may prevent or inhibit the development of fully mature, large follicles. This is indicated by several of our experiments and observations, which show that a reduction of luteinization processes may bring out more prominently maturation processes. It can be readily understood that premature maturation of the granulosa, luteinization of theca interna, as well as early ingrowth of connective tissue and blood vessels into the changed granulosa are not favorable to the full growth of follicles and their subsequent maturation (Loeb et al., '36). 2) There is noticeable the antagonism between the effects of the maturation and luteinization hormones on the proliferation of the vaginal epithelium, which we have discussed on previous occasions. Processes of luteinization inhibit the full development of squamous epithelium in the vagina (Loeb, '23).

What the character of the changes is which takes place in the human anterior pituitary gland at different periods of life is largely unknown; we know however that they are reversible, and we know furthermore that during pregnancy structural modifications take place in the anterior pituitary

gland, which presumably are related to the changes in the effects produced by this gland. It is furthermore of interest that it is the maturation hormone which persists longest and is least readily interfered with under different conditions of life. We observed furthermore that luteinization processes of the first type may appear more readily than luteinization processes of the second type, in which theca luteinization predominates, although as a rule the various luteinization processes are combined. This sequence is the opposite of what we find in experiments in which the anterior pituitary glands of different species are subjected to various experimental interferences in vitro. If this is done, it is usually the luteinization processes which predominate while it is much more difficult to obtain pure maturation processes. Correspondingly, it is presumably the relatively considerable intensity of the luteinization processes which are induced by the human anterior pituitary gland, which obscures to some extent the presence of the follicular maturation producing agent under ordinary circumstances after implantation of the adult organ.

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THE RATE OF UTERINE GROWTH RESULTING FROM CHRONIC DISTENTION¹

SAMUEL R. M. REYNOLDS² AND SANFORD KAMINESTER³

Department of Physiology, Long Island College of Medicine, Brooklyn

THREE FIGURES

Certain growth changes take place in the uterus of an untreated, ovariectomized rabbit when this organ is distended with a pellet of suitable size. It has been found (Reynolds and Kaminester, '36) that the pellet should be more than half yet less than twice the size of the uterus in order that appreciable growth takes place. The largest growth response is obtained when the pellet and uterus are of approximately equal sizes. These relationships were ascertained for a distention period of 2 weeks, commencing 1 week after ovariectomy. No evidence was obtained on the rate at which the growth responses in the uterus take place, nor was it ascertained that growth was complete at this time. The present experiments furnish this information.

PROCEDURES

The experiments were of two types. In the first group, two or three paraffin pellets (m.p. 56°) were inserted per vaginam into the uteri of mature rabbits in the manner described before (Reynolds and Kaminester, '36). At stated times after insertion of the pellets the distention sites were taken, fixed and studied, both for the histological features and the amount of

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² Fellow of the John Simon Guggenheim Memorial Foundation.

³ Member of the Department of Obstetrics and Gynecology, Long Island College of Medicine.

increase in gross size of the tissues. Comparison of these changes in the distention sites was made with segments of untouched, undistended parts of the corresponding uteri. The times at which the tissues were taken after insertion of the pellets were a) immediately after distention (acute distention); b) 24 hours after distention; c) 4 days and d) some additional distention sites were taken on the fourteenth day in order to confirm and extend the earlier series (Reynolds and Kaminester, '36). The method by which the growth resulting at the distention sites was estimated has been described before. Similarly, the method by which the degree of distention was ascertained has been reported; briefly, it consists of determining the ratio of the cross sectional area of the undistended part of a uterus (including its lumen) to the cross-sectional area of the distended pellet. Thus, a 1:1 (1.0) distention ratio means that the uterus and pellet are of equal cross sectional areas; a 2:1 (2.0) ratio means that the uterus is twice the size of the pellet and a 1:2 (0.5) ratio, that the uterus is one-half the size of the pellet. The size in each case is determined by planimeter measurements of camera lucida drawings of average cross sections.

The second group of experiments differed from the first in the method of distention employed and were performed in order to obtain information regarding the capacity of the uterus of the untreated, ovariectomized rabbit to become adapted to progressively increasing distention, as the uterus must do in the more complex state of pregnancy. Instead of using paraffin pellets of constant size as in the first group of experiments, fluid was allowed to accumulate in the lumen of the uterus between two ligatures placed about an inch apart. In this way, the uterus was progressively distended, although the rate at which fluid accumulated is not known. After 4 days, the distended segment (along with an undistended portion of uterus for control purposes) was fixed with the fluid in situ. It was possible to ascertain the end amount of distention in each instance, and of course the growth increments in the tissues were measurable as before.

RESULTS

Acute distention. As noted already, the histological features associated with acute distention show that there is considerable loss of fluid and blood from the tissues about the pellet. With the larger degrees of distention, the endometrium is reduced to a thin layer in which the epithelium rests upon the myometrium in some places. The myometrium is thin and avascular. Rarely, small hemorrhages may be seen, but otherwise there is little evidence of trauma. Measurements from six sites at which the distention ratio was 1.0 or less show that a reduction in size of the tissues of 18 to 35% takes place. The immediate effect of distention, therefore, is to force blood and tissue juices from the distention sites.

Twenty-four hours after distention. Figure 1 shows that by the twenty-fourth hour or thereabout after insertion of the pellets, eleven out of fourteen distention sites regained or exceeded by a little their original size, as gauged by comparison with untouched, undistended parts of the same uteri. The impression that one obtains from studying these tissues under the microscope is that the changes which take place during the first 24 hours of distention consist of a restitution of the fluid matrix of the tissues; ordinary histological methods fail to show any sign of cellular growth such as may be demonstrated on the fourth day and later, as described below.

Distention for 4 days and longer. In figure 2, the percentage increases in size of the various distention sites after 100 hours of distention are shown (triangles), along with the growth after 14 days of distention (dots). These points, through their manner of distribution, show that by the fourth day of distention, the size of the tissues about the pellets is as large as it will become, with this mode of distention. At least, no increase in size is found to take place between the fourth and the fourteenth days of distention. In confirmation of the earlier data (Reynolds and Kaminester, '36), the present results show that the pellets are effective stimuli for growth when they are more than half the size of the undistended uteri, and that maximal growth responses are obtained when the pellet and

the uterus are of approximately equal sizes. With still larger degrees of distention, the pellets are less and less effective as growth-promoting stimuli. The reasons for this have been discussed elsewhere (Reynolds and Allen, '37).

In addition to the fact that the gross enlargement of the tissues is achieved by the fourth day after distention is the further fact that the histological changes resulting from insertion of the pellets are also completed by this time. The

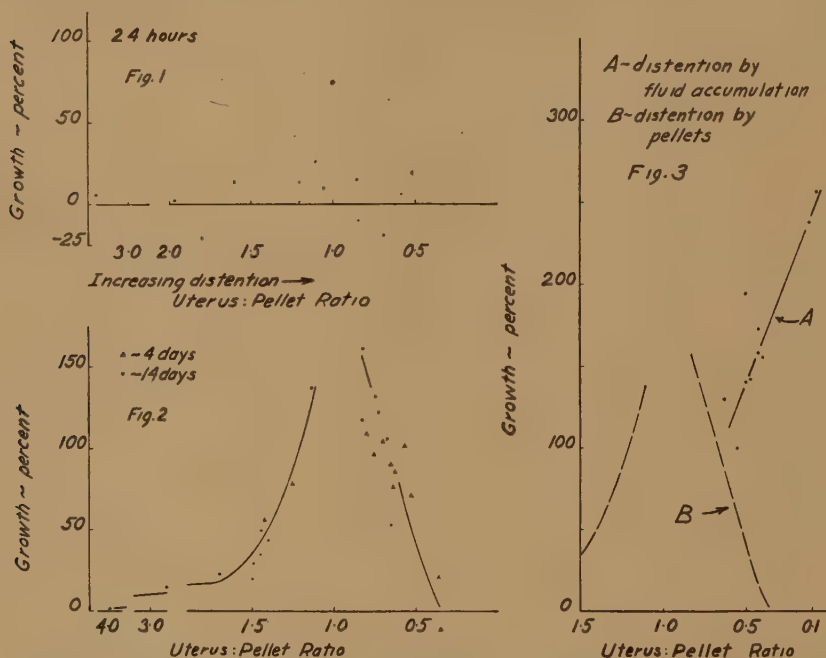


Fig. 1 Chart showing the size of distention sites relative to non-distended sites of rabbit uteri, expressed as percentage of the latter. Correlated with the amount of distention in each case. A distention ratio of 2.0 means that the uterus was twice the size of the pellet; of 1.0, the uterus and pellet were of equal size; of 0.5, the uterus was half the size of the pellet. Similarly for other figures. Time of distention, 24 hours.

Fig. 2 Chart showing growth of chronically distended uteri after 4 days (triangles) and 14 days (dots). Paraffin pellets were used, $\frac{3}{8}$ inch in length and of known, uniform diameters. No increase in size of uteri between the fourth and fourteenth days. See text for description of histological features.

Fig. 3 Chart showing the effect of progressive, increasing distention (curve A) by accumulation of fluid in the lumen of uteri; distention period, 4 days. Curve B, redrawn from figure 2.

cells of all parts of the tissues about the pellets undergo marked hypertrophy, as shown by an increase in ratio of cytoplasmic to nuclear material; the nuclei become vesicular; the vascular bed is somewhat increased; the tissues are edematous, especially in the deeper portions of the endometrium, and hyperplasia, even of the smooth muscle (Allen, et al., '37), takes place.

These results show, therefore, that the initial response of the uterus to distention is to replace the tissue fluids lost as a result of insertion of the pellets. Then, according to the effectiveness of the degree of distention, growth changes are evident in all the tissues. These structural changes are largely complete in less than 4 days, so far as may be judged by these experiments. Evidence has been presented elsewhere which shows that the essential nature of the distention stimulus is the creation of an appropriate increase in tension upon the tissues (loc. cit.), but if the tension becomes too great, the nutrition of the tissues may be impaired through impoverishment of the blood supply.

Progressive distention by accumulation with fluid. In this group of experiments the least amount of distention obtained exceeded a uterus: pellet ratio of 1:1.59 (distention ratio of 0.63) or, as shown in figure 3, a point far down on the right of curve B, redrawn from figure 2. In contrast to curve B, curve A of figure 3 shows that the growth responses increase in amount as the distention increases, as far as the data go. The greatest growth response was one of 255% increase, or a three and a half-fold increase, at a distention ratio of 1:12.5 (i.e., the pellet was $12.5 \times$ the size of the undistended uterus). This is the largest amount of stretching obtained thus far in untreated, ovariectomized rabbits, and was possible only because the uterus grew as it became increasingly distended. Clearly, therefore, when the uterus is progressively distended it is capable of both rapid and extensive adaptive alterations in structure *pari passu* with the increasing distention.

Mention may be made in passing of the extent of uterine growth which takes place in the rabbit during pregnancy.

Hammond's data ('35) on this subject show that a five- to eightfold increase takes place. This figure compares favorably with that stated by Stieve ('32). It is striking, therefore, that the three and a half-fold increase that was observed in the instance cited above took place in 4 days, and in the absence of ovarian or other hormones associated with gestation. The role of distention during gestation has been discussed in two other places (Reynold's, '37 a, b).

SUMMARY

The rate of the changes in uterine structure and size resulting from chronic uterine distention is described. It is found that the growth changes, including both hypertrophy and hyperplasia, even of smooth muscle, are completed in less than 4 days after insertion of paraffin pellets of uniform and known sizes. It is further observed that when distention is achieved by progressive accumulation of fluid within the uterus the growth response is proportional to the degree of distention, as far as the data go. It is further found that the percentage increase in size of the uterus of the untreated, ovariectomized rabbit under the latter conditions approaches in some instances that which takes place during normal pregnancy in the rabbit.

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THE OCCURRENCE OF ARTERIO-VENOUS ANASTOMOSES IN THE TONGUE OF THE DOG

MARGARET E. BROWN

Department of Anatomy, Cornell University Medical College

TWO PLATES (FOUR FIGURES)

The study of direct communications between arteries and veins (arterio-venous anastomoses) has aroused considerable interest in recent years. These arterio-venous connections are now known to be of widespread occurrence in mammals. A detailed account of their distribution and structure in birds and mammals has been given by Max Clara ('27).

As far as I am aware, arterio-venous anastomoses have never been seen in the tongue of any of the laboratory animals, and a positive statement to the effect that they do not occur in the rabbit's tongue is made by Vastarini Cresi (quoted by Clara, '27). Work in this laboratory on the nerve supply of the tongue of rabbits and cats has never disclosed any of these structures; however, their presence had been suspected in the dog's tongue by Dr. J. F. Nonidez. His attention was first attracted by the abundance of thick, apparently sensory fibers, present in the adventitia of certain arterial segments with modified walls. At his suggestion the study of these fibers and the arterio-venous anastomoses which they supply was undertaken.

It was found that the anastomoses in the tongue of the dog may occur in the tunica propria of the mucosa lining the dorsal surface of the tongue, or more deeply among the muscles underlying the lingual fascia. They are probably more numerous near the edge of the tongue than toward the center of the dorsal surface, but no study of their frequency and

distribution was attempted. As compared with the arterio-venous anastomoses described by Masson ('35) in the human, those found in the dog's tongue are relatively simpler though they agree in their fundamental characteristics. It is clear, however, that they are not as numerous as in the human nail bed, for some sections of the edge of the tongue may not show any of these structures. The nerve fibers branching around the arterio-venous anastomoses are of two types: 1) Very numerous, probably unmyelinated fibers, surrounding the anastomotic segment. 2) Much thicker, apparently afferent fibers, with massive adventitial terminations on the segment and in the immediate vicinity.

Two silver impregnation methods were used: 1) The Cajal technique for the staining of nerve fibers and terminations in frozen sections (40 to 45 μ) of formalin preserved material. 2) The Cajal method for impregnation of blocks after fixation with chloral hydrate, followed by hardening and alkalization with alcohol ammonia. The latter material was embedded in paraffin and cut serially at 12 μ .

Our knowledge of the histology of the arterio-venous anastomoses has been considerably advanced by the contributions of Max Clara ('27), Grant ('30), Grant and Bland ('31), Popoff ('34, '35) and Masson ('35, '36).

Three different segments can be distinguished in the more differentiated arterio-venous anastomoses: 1) The afferent artery, which may give rise to from one to four separate connecting canals (Sucquet-Hoyer canals) and which is unmodified except near the origin of the canals where it has a relatively stout adventitia and numerous muscle cells. 2) The collecting vein, collecting blood from the Sucquet-Hoyer canal and from arterioles which nourish the constituents of the anastomoses. Though lacking in musculature the vein is richly supplied with elastic fibers. It is in no way specialized, but being long and wide it may form a voluminous receptaculum almost encircling the Sucquet-Hoyer canals and having a wide surface area. 3) The Sucquet-Hoyer canal proper, through

which the blood may pass from the artery into the vein without traversing any intervening system of capillaries. It is marked from its beginning by the presence of clear endothelio-muscular cells, involving a considerable thickening of the adventitial and muscular coats. The muscle cells are shorter and their nuclei rounder here than in the artery. There is no elastic tissue present in the wall of the canal, and its lumen appears small and irregular.

The Sucquet-Hoyer canal is usually surrounded by a clear, wide zone which may be considered as an expansion zone consisting of loose, fine collagenous reticulum enclosing a rich network of nerve fibers.

The canal is the principal feature of the anastomoses and because of its characteristic modifications, arterio-venous anastomoses are readily identified even when all their diverse portions are not included in the section.

In the material studied by me the Sucquet-Hoyer canal could be identified in the frozen sections stained with the Cajal technique (figs. 1, 3 and 4) even better than in the paraffin sections, since there was little or no shrinkage. In serial sections, however, it was possible to trace the connections between the artery and the vein. With both techniques the nerves appear stained deep black.

The complexity of the nerve endings around the anastomoses may be such that they obscure the canal, and under such conditions it is difficult to recognize the diverse segments of the anastomoses. This is seen in figure 1 copied from a preparation of formalin preserved material. Since the section was quite thick (45μ) a Sucquet-Hoyer canal (c) appears intact, only the ends having been cut by the knife. The cell elements of the canal do not appear stained in the section; however, its identification and its relation to the anastomoses was clearly seen because of the very large number of nerve fibers which surround the canal and do not occur in the arteries. At the left a rather large bundle of small and medium-sized unmyelinated fibers (n) is seen entering the capsular portion. Parts of two large thick myelinated fibers are also present.

The relatively large reticulated endings (e) are terminations of the thick fibers. The thick and thin fibers closely encircle the canal, forming a rather dense network around it. A complete representation of all the fibers seen around the canal was not attempted in order to avoid confusion.

In cases in which an anastomosis appears included in several sections it is possible to ascertain more accurately the location of the branches of the nerve endings, even though its aspect is not so impressive as when it has not been sectioned by the knife.

Figure 2 represents a portion of an anastomosis which appears in six sections. The area selected for drawing shows the Sucquet-Hoyer canal in cross section (c), the artery from which it springs (a) and a section of the vein into which the Sucquet-Hoyer canal opens (v). Only a portion of the thick fiber forming the afferent (sensory) termination appears in the figure. In this case the fiber was traced to a bundle running in the tunica propria. Some of the branches of its arborization as well as a bundle of nerve fibers (n) reaching the anastomosis with the artery are also seen. In the canal of Sucquet-Hoyer the characteristic appearance of the nuclei of the media is readily recognized; they are round or elliptical and contain few chromatin granules. Some of the nerve branches enter this coat of the anastomosis but for the most part the branches of the afferent fiber end in the adventitia.

Figure 3 shows a double Sucquet-Hoyer canal in cross section. Both sections of the canal are enclosed in a common sheath of connective tissue. In the same field is seen the artery (a) which probably gives rise to these canals. The cushion-like appearance of the media and the large, oval nuclei of the modified muscle cells are highly characteristic. In the adventitia can be seen portions of relatively thick branches of an afferent fiber. Two bundles of fine unmyelinated fibers (n) also occur in the figure. Since the arborization of the thick fibers is very extensive only a small portion of it appears in the section, even though the latter was cut 45μ thick. Some of the twigs end in minute rings or in small club-shaped enlargements with reticulated structure.

Figure 4 represents a section passing through the afferent artery near the point of emergence of the Sucquet-Hoyer canal. Fine collagen fibers, so typical of arterio-venous anastomoses, enclose the artery in a loose whorl and are continuous with its adventitia. The muscle coat is somewhat thickened but it is obvious that the modification of the cells of the media and intima is less marked than in the canal proper. The branches of a sensory fiber in this case have a clearly reticulated aspect due to a loosening of the neurofibrils. Since the section is slightly oblique they seem to end on the media.

The presence of thick myelinated fibers ending on the arterio-venous anastomoses has already been reported by Masson ('36). He regards these terminations—which in the skin are derived from bundles in the dermis—as tactile nerve endings. Judging from the aspect of the arborizations of the thick fibers in the dog's tongue, one might agree with the opinion expressed by Masson. However, in the tongue there are striated muscle fibers which, during contraction, might tend to reduce the lumen of the Sucquet-Hoyer canal passively as well as the vessels it connects. There is, therefore, the possibility that the nerve endings in this case are some sort of mechanoreceptors affected by muscular contraction. It is also possible that the branches of the afferent fibers are stimulated by the opening of the Sucquet-Hoyer canal upon the passage of the blood from the artery to the vein.

In any event, it seems reasonable to conclude that the elaborate arborizations of the thick fibers are receptor mechanisms primarily concerned with vascular reflexes in which the activity of the arterio-venous anastomoses is involved. On the other hand, the much thinner, probably unmyelinated fibers may be concerned with vaso-constriction since they end on the surface of the modified media of the Sucquet-Hoyer canal. When we bear in mind that the dog lacks sweat glands and that he thrusts the tongue out of the mouth when the temperature of the body rises, one is tempted to believe that the arterio-venous anastomoses present in the tongue of this animal may be connected in some way with the elimination of heat from

the body. It is possible that the anastomoses remain open while the tongue is held within the mouth, and that they close when it is thrust out, thus increasing the circulation in the capillary bed and favoring the irradiation of heat.

SUMMARY

1. Typical arterio-venous anastomoses are described in the tongue of the dog.

2. These anastomoses, while not so complex as those described in other parts of the body, have an extremely rich nerve supply.

3. This nerve supply consists of: 1) Thin unmyelinated fibers with terminations in the media of the canal of Sucquet-Hoyer and 2) thick myelinated fibers with terminations mainly located in the adventitia.

4. The thick fibers are regarded as afferent. They are probably concerned with vascular reflexes affecting the arterio-venous anastomosis in which they occur.

5. It is suggested that the presence of arterio-venous anastomoses in the dog's tongue may be connected in some way with the elimination of heat upon a rise of the body temperature.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

With the exception of figure 2 all figures were copied from sections of the dog's tongue prepared with the Cajal technique for frozen sections of formalin preserved material. Figure 2 was copied from a section impregnated with the silver nitrate method of Cajal. The drawings were outlined with the camera lucida using objective 40 and ocular $\times 20$ on a Zeiss binocular monobjective microscope; c, canal of Sucquet-Hoyer; e, sensory nerve endings; a, artery; v, vein; n, nerve.

1 Complete canal of Sucquet-Hoyer showing elaborate innervation. Cell elements of vessel not stained.

2 Cross section of a Sucquet-Hoyer canal, the artery from which it springs and the vein into which it opens in slightly oblique section. Portion of a thick sensory fiber and sensory terminations are seen in the adventitia; n, nerve bundle reaching the anastomosis with the artery.

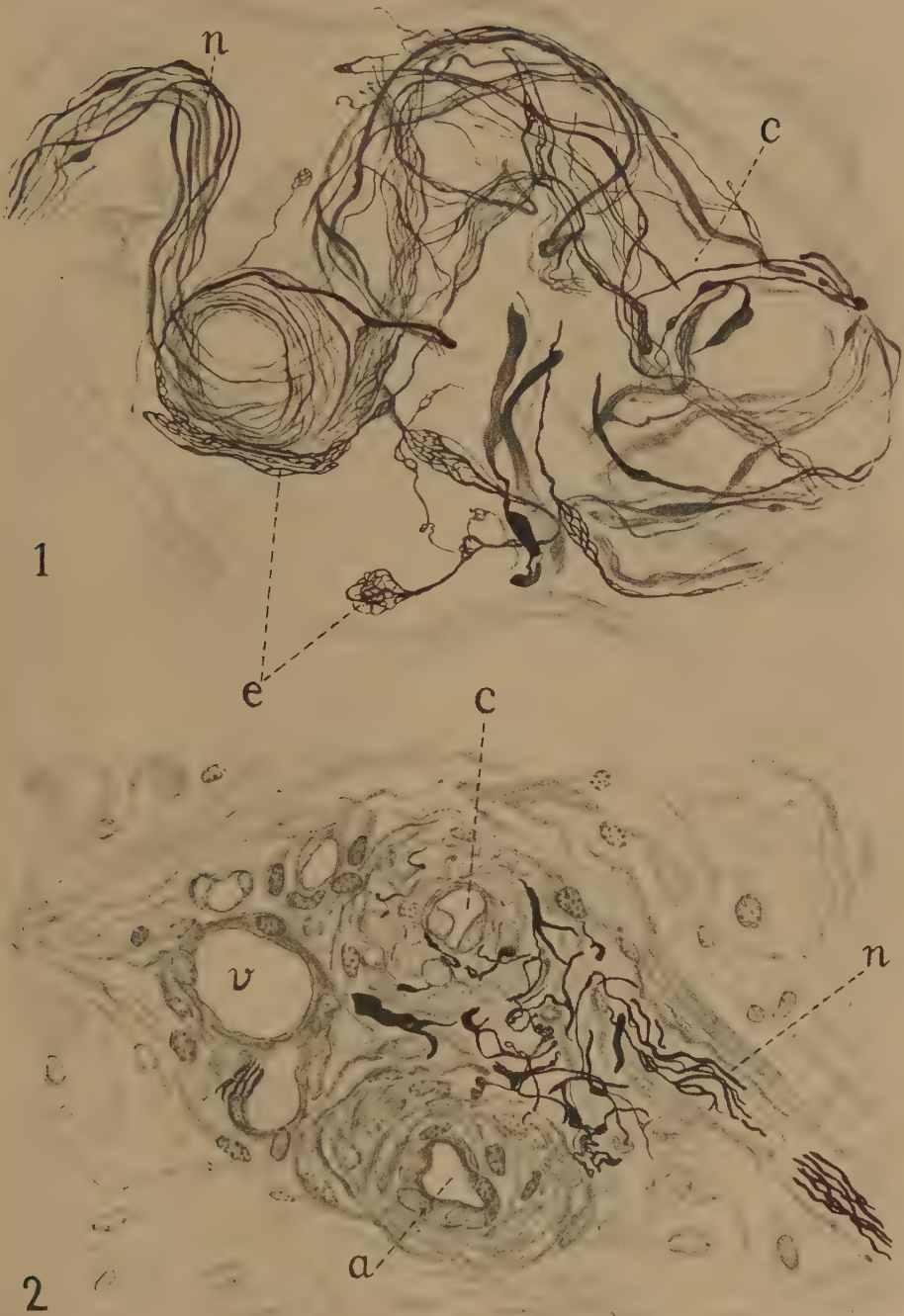
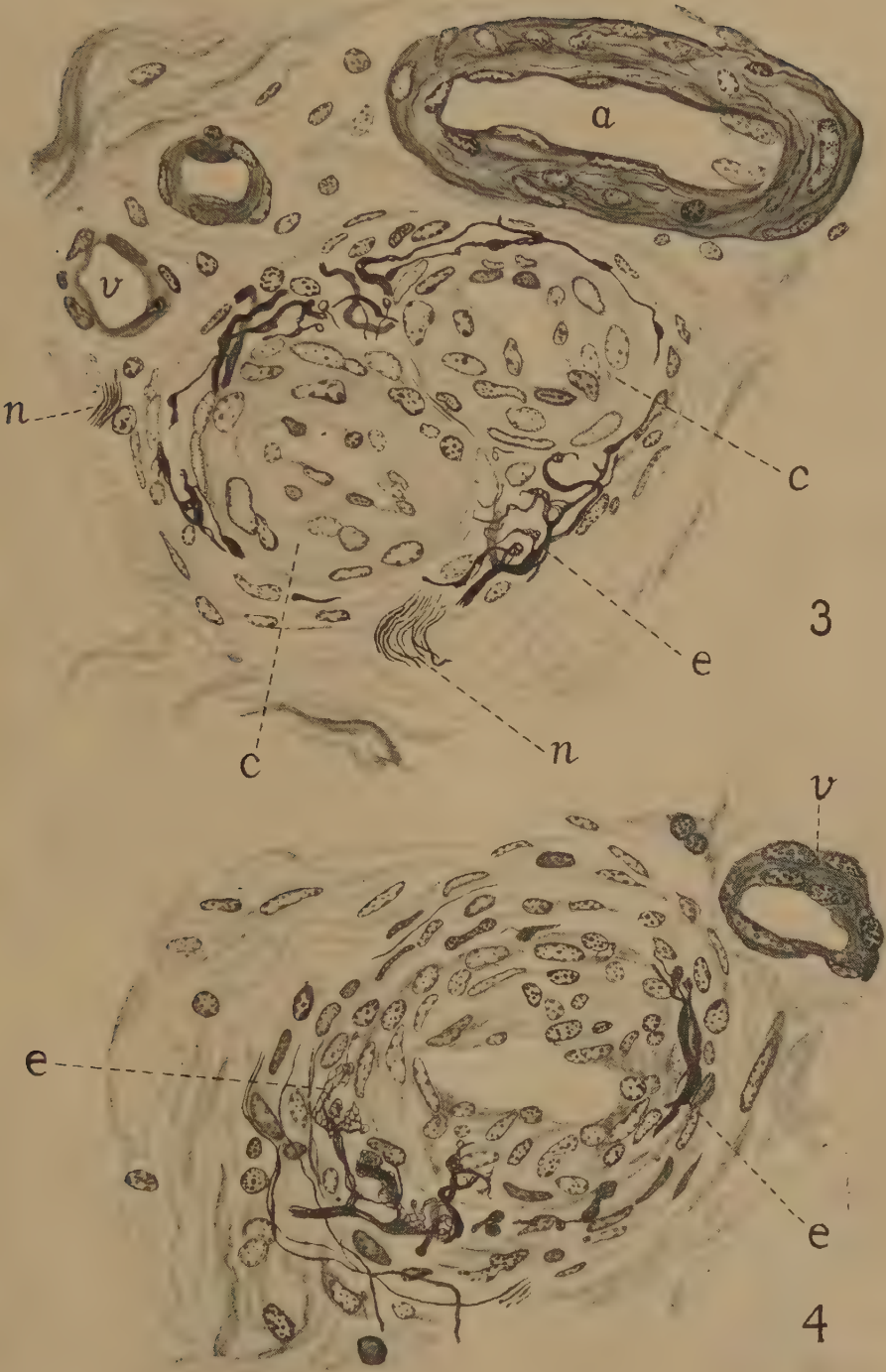


PLATE 2

EXPLANATION OF FIGURES

- 3 Double Sucquet-Hoyer canal in cross section. Fine unmyelinated (n) fibers and portions of thick branches of afferent fibers present in the adventitia.
- 4 Slightly oblique section of afferent artery near an arterio-venous anastomosis. A branch of a sensory fiber enters the adventitia.



DISTRIBUTION OF THE AORTIC NERVE FIBERS AND THE EPITHELIOID BODIES (SUPRACARDIAL 'PARAGANGLIA') IN THE DOG

JOSÉ F. NONIDEZ

Department of Anatomy, Cornell University Medical College

TWO TEXT FIGURES AND TWO PLATES (FIVE FIGURES)

The present article is based on studies of frontal serial sections of the hearts of two puppies prepared with the silver nitrate method of Cajal. Similar studies in the guinea pig, rabbit and cat have been previously reported (Nonidez, '35). It was found in these animals that the aortic (depressor) fibers ending as pressoreceptors are accompanied by thinner fibers which terminate in epithelioid bodies structurally identical with the carotid glomus. These bodies, first described by Muratori ('34, '35), were termed the aortic glomus. In addition there are other masses of epithelioid cells located between the ascending aorta and the pulmonary artery. They have been designated by Penitschka as the paraganglion aorticum supracardiale. Since the position of the epithelioid bodies in the dog is different from that of the corresponding structures of the rabbit and cat they will be considered in this paper.

With the fixation used (25% chloral hydrate followed by hardening and alkalization with alcohol-ammonia) it is possible to distinguish the following types of fibers in the nerves that supply the heart: 1) Thick fibers stained dark brown to black, enclosed within myelin sheaths. 2) Numerous closely placed, deeply stained fibers of medium and small caliber. Since the distances involved in the hearts of newborn puppies are very small it is possible to trace many of these

fibers to their destination. A considerable number of them are parasympathetic preganglionics ending as pericellular baskets in the ganglia of the deep cardiac plexus. Similar fibers, some of which are of fairly large size, reach the epithelioid bodies and branch among their cells. 3) A third category comprises thin, faintly stained fibers which abound in the sympathetic ganglia and are seen coursing in large numbers in the nerves issued from them; these are the sympathetic postganglionics. The three types of fibers can be seen in figure 4.

The differences noticed in the staining of the fibers within the cardiac plexus indicate a varying degree of affinity for the silver, due perhaps to slight differences in the chemical composition of the fibers or in the physical state of their constituents. In this connection it is interesting to note that the postganglionics issued from the ganglia of the cardiac plexus stain deeply even though in many cases they are not thicker than the sympathetic postganglionics. The varying degree of affinity for the silver observed in the nerve fibers of the dog is also encountered in those of other animals when chloral hydrate is used for fixation.

In regard to the photomicrographs of figures 3, 4 and 5, it must be stated that they are intended to show the relative sizes of the nerves which carry aortic nerve fibers and to some extent too their composition. However, since the nerves were not dissected out and straightened for sectioning, and since the sections were cut thick (15μ) many of the fibers appear in oblique view. Paraffin was used for embedding, the higher alcohols and the clearing agent being eliminated through the use of dioxan.

All figures are from the heart of a day-old puppy (Pekingese $\times$ Dachshund) selected because of its small size. The other heart studied was from a week-old hybrid of larger breeds (Bassethound $\times$ German Shepherd). I am indebted to Prof. Charles R. Stockard for this material.

COURSE AND DISTRIBUTION OF THE AORTIC (DEPRESSOR) FIBERS

The dog lacks an independent aortic (depressor) nerve. The aortic fibers form a bundle which courses in the vago-sympathetic trunk. In some instances the bundle cannot be easily dissected out from the sympathetic (Heymans and Bouckaert, '33). The aortic bundle arises typically from two roots, one from the vagus, the other from the superior laryngeal nerve, but instances in which there are three roots instead of two have been observed (Nonidez, '31, fig. 1). The aortic fibers leave the vago-sympathetic trunk as the latter reaches the inferior cervical ganglion, joining the cardiac nerves issued from the vagus at this level. As is the case in other animals studied, the left aortic bundle is larger than the right since it carries more afferent fibers some of which enter a nerve issued from the inferior cervical ganglion (figs. 1 and 2, cs).

Right aortic nerve. The bundle of aortic fibers on the right side (figs. 1 and 2, ab) enters the right cardiac nerve through an anastomosis (ca) which carries postganglionics from the inferior cervical ganglion (ic). The afferent aortic fibers course within the cardiac nerve (cn) for some distance, and enter a small branch given off by this nerve. This may be called the aortic nerve (an), which is distributed over the distal portion of the innominate artery and the base of the subclavia. It corresponds to a branch of similar distribution represented by Schurawlew ('28) in his figures on the innervation of the dog's heart, based on dissections in which the nerves had been previously stained with a modified methylene blue technique.

A cross section of the right aortic nerve is seen in figure 3, an. The bulk of its fibers are sympathetic postganglionics—contributed by the inferior cervical ganglion—which join the extensive plexus occupying the externa of the innominate and the subclavia; the latter also receives fibers from the ansa subclavia (as) and the inferior cervical ganglion (ic). Thick and medium-sized fibers occur scattered or forming small groups among the bundles of postganglionics; no more than thirty were counted in the nerve photographed. Most of the medium-sized fibers end among the cells of the small, poorly

developed aortic glomus of this side (fig. 2, ag). The larger, myelinated fibers are distributed chiefly on the lateral surface of the innominate-subclavia junction, as shown in the diagrams. The nerve arborizations are not numerous but they are quite extensive and elaborate, and agree in all respects with those present in the cat and rabbit. However, the large, reticulated enlargements seen in the two animals just mentioned are represented in the dog by more discrete swellings and the same applies to the pressoreceptors occurring in the walls of the venae cavae and the pulmonary veins, considered in another article ('37).

A portion of the right cardiac nerve also appears in figure 3, cn; the point of emergence of the aortic nerve has been indicated by *. The right cardiac nerve carries numerous sympathetic postganglionics, deeply stained preganglionics and probably too fibers for the epithelioid cell masses located between the aorta and the pulmonary trunk. There are also thick myelinated fibers, presumably afferent. In the puppy selected for illustration typical monopolar cells of sensory type occur in the cardiac nerve below the exit of the aortic nerve; their peripheral prolongations follow the cardiac nerve on its way to the heart. The presence of groups of migrated sensory neurones below the ganglion nodosum of dog fetuses has been reported by M. E. Brown ('36).

Left aortic fibers. Strictly speaking there is no left aortic nerve in the dog, for the fibers ending in the pressoreceptor area of the arch of the aorta reach their destination through at least two different routes. Upon reaching the level of the

Fig. 1 Diagram showing the distribution of the nerves bearing aortic fibers on the large arteries of the heart. Bundles of aortic fibers shown in black; epithelioid cell groups, stippled. One-day-old puppy. a, main pressoreceptor area; a', pressoreceptor area at the bifurcation of the pulmonary trunk; ab, ab', bundles of aortic fibers in the vago-sympathetic trunks; an, right aortic nerve; as, as', ansae subclaviae; ca, ca', anastomoses between the inferior cervical ganglia (ic, ic') and the cardiac nerves (cn, cn'); ca'', anastomosis between the left cardiac nerve and the left recurrent, r'; cs, cardio-sympathetic nerve; g, epithelioid cell mass corresponding to the left aortic glomus; pg, bundle of preganglionics and fibers to the epithelioid bodies under the arch of the aorta; r, r', recurrent nerves; s, s', stellate ganglia; v, v', vagi.

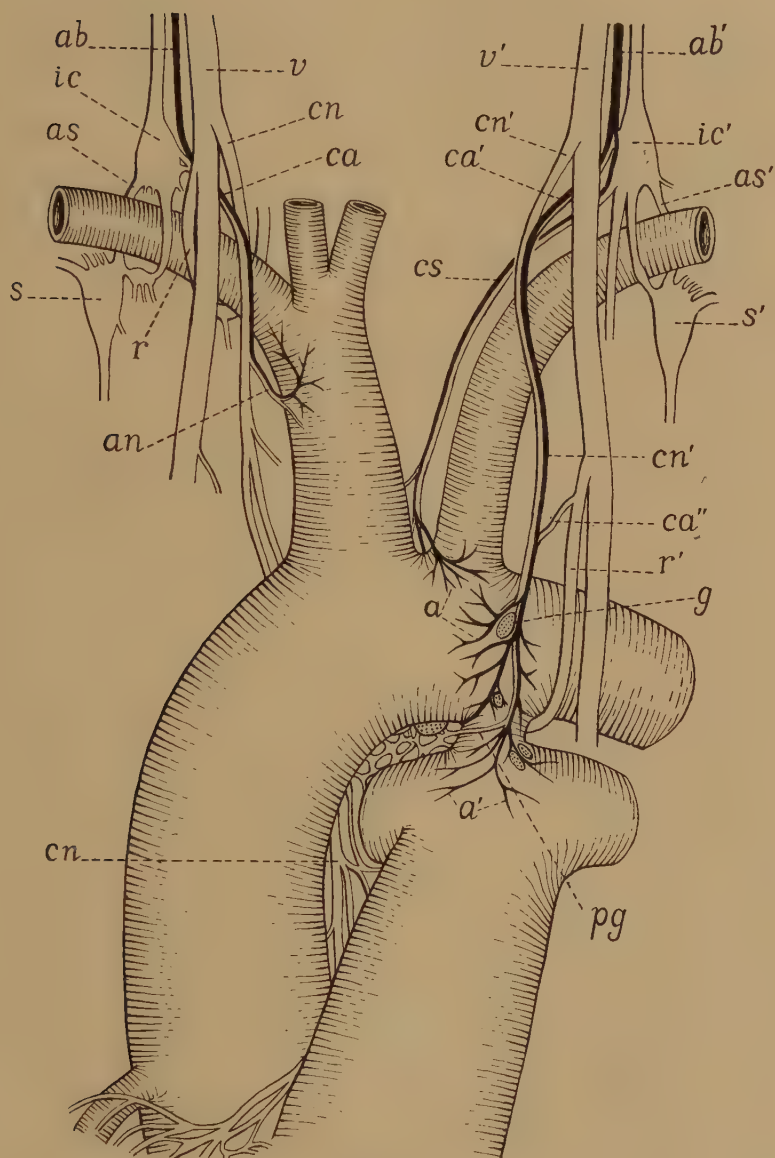


Figure 1

inferior cervical ganglion the fibers of the left aortic bundle (figs. 1 and 2, ab') join an anastomosis (ca') between this ganglion and the left cardiac nerve (cn') except for a number of them which enter the ganglion to emerge with a bundle of postganglionics running as a thin nerve (cs) between the innominate artery and the left subclavia. This cardio-sympathetic nerve was present in the two hearts examined.

The left cardiac nerve (cn') carries, beside the thick aortic components, numerous deeply stained fibers which form a distinct bundle on one side of the nerve (fig. 4, p). These fibers are of two kinds, namely: a) Thin preganglionics for the small ganglia of the plexus located between the arch of the aorta and the pulmonary trunk (fig. 6), and b) thicker fibers ending in epithelioid cell masses occupying the same territory (fig. 2, g). The preganglionics and fibers for the epithelioid cells arrange themselves in a much flattened bundle which runs on the anterior surface of the ductus arteriosus and reaches the plexus mentioned before (fig. 1, pg). The other fibers in the nerve are sympathetic postganglionics (fig. 4, s).

In frontal sections the left cardiac nerve is seen to run mesial and close to the vagus from which it may receive a few anastomoses; in the puppy on which the diagrams are based one of these anastomoses actually arises from the left recurrent (figs. 1 and 2, ca'', and 4, a). Upon reaching the anterior (ventral) surface of the arch of the aorta the cardiac nerve breaks into branches which enter the externa of this vessel (fig. 1, a). The thin, sympathetic postganglionics coursing in the nerve follow these branches and contribute to the formation of an elaborate adventitial plexus for the supply of the smooth musculature of the aorta. A particularly stout bundle of sympathetic postganglionics courses between the arch of the aorta and the pulmonary, sending branches to both vessels.

The aortic fibers within the left cardiac nerve reach the externa of the aorta through the branches mentioned above, and end as pressoreceptor arborizations in the externa and the outer third of the media. The nerve, however, does not

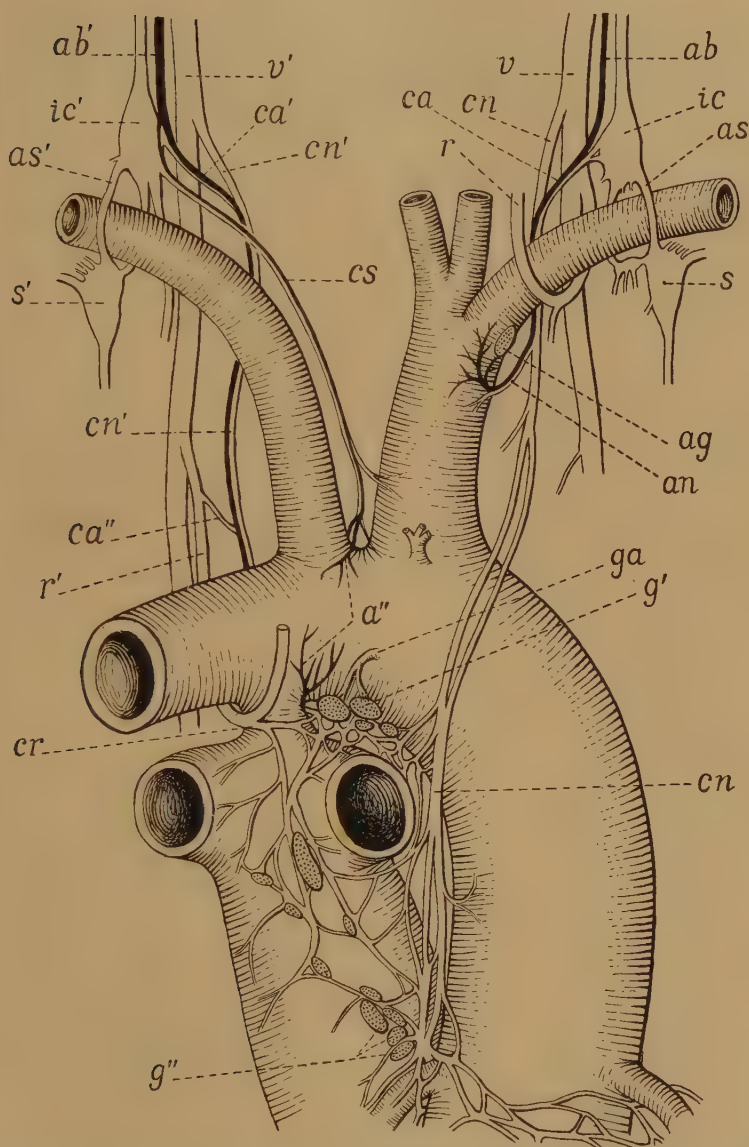


Fig. 2 Posterior view of the aorta and the pulmonary trunk. *a''*, aortic fibers ending on the posterior surface of the arch of the aorta; *ag*, epithelioid cell group (right aortic glomus); *cr*, cardiac branch of the left recurrent nerve; *g'*, *g''*, superior and inferior groups of epithelioid bodies, respectively; *ga*, artery to superior epithelioid bodies. Other letters as in preceding figure.

end in this vessel, for some of the aortic fibers run on the anterior surface of the ductus arteriosus and are distributed over the anterior aspect of the bifurcation of the pulmonary trunk, forming a second pressoreceptor area (a'), not present in the rabbit and cat although in these animals there are pressoreceptor endings in the wall of the ductus arteriosus. A good number of the sympathetic postganglionics in the nerve continue below the arch of the aorta as a bundle which joins the plexus between this vessel and the pulmonary; the bundle carries aortic fibers which end on the posterior (dorsal) surface of the arch of the aorta. Finally, most of the deeply stained, medium-sized and small fibers coursing in the cardiac nerve form the flattened bundle already mentioned as joining the plexus between the aorta and the pulmonary (fig. 1, pg).

The sympathetic nerve carrying aortic fibers (figs. 1 and 2, cs, and fig. 5) is smaller than the cardiac nerve. As already stated, it runs in the space between the innominate artery and the left subclavia, and it sends branches to both arteries. Upon reaching the arch of the aorta it breaks into several branches, the largest of which ends at the base of the left subclavia. Together with the branches issued from the left cardiac nerve it contributes to the formation of the pressoreceptor area of the arch of the aorta (fig. 1, a). Another branch reaches the posterior surface of the latter, where it ends, usually not far from the base of the subclavia. In cross section the nerve under consideration is seen to consist chiefly of sympathetic postganglionics (fig. 5, s), among which appear scattered thick and medium-sized aortic fibers.

Although the two nerves described are the main paths for the left aortic fibers it is probable that some of the latter also course in the vagus and reach the aorta indirectly through the cardiac branch of the recurrent (fig. 2, cr), for this branch joins the plexus between the pulmonary and the arch of the aorta. This contribution, however, must be small.

As to the extent and location of the aortic pressoreceptor area of the dog it may be said that it has a crescentic shape, with the zone of maximum density of nerve endings occurring

in the anterior surface of the aorta instead of the supero-posterior, as is the case in the rabbit. The nerve endings do not differ from those already mentioned in connection with the right aortic nerve, and although they may increase in size and complexity during postnatal growth they probably do not extend much beyond the area they occupy in the newborn. Pressoreceptor nerve endings were not found in the ascending aorta of the two puppies studied, nor were they present in the descending portion of this vessel. This is in agreement with the findings in the guinea pig, rabbit and cat. Diagrams in current textbooks showing a much wider distribution of the aortic nerves require correction in view of the histological observations of several workers (Tello, Tschernjachiwsky, Muratori, Nonidez).

POSITION AND STRUCTURE OF THE EPITHELIOID BODIES

Groups of epithelioid cells ('paraganglia') closely resembling the elements of the carotid glomus occur in various locations in the heart of the dog, but their position differs from that of the corresponding structures of the rabbit and cat.

As already stated, in the cat and the rabbit two groups of epithelioid cells occur above the arch of the aorta. One of them (left aortic glomus) occupies the space between the bases of the innominate artery and the left subclavia, respectively; the other (right aortic glomus) occurs in the angle formed by the right subclavia and the right common carotid. In the two animals mentioned the aortic (depressor) nerves course caudad toward the heart, and they convey the fibers ending among the cells of each aortic glomus. In the rabbit the aortic fibers run as an independent nerve on each side of the neck, receiving sympathetic postganglionics from the inferior cervical ganglion. On the right side the aortic nerve ends on the subclavia—the right fourth aortic arch of the embryo—while on the left it reaches the supero-posterior surface of the arch of the aorta where it ends, having previously sent fibers to the aortic glomus of this side. The aortic nerves of the cat are shorter and less distinct, leaving the vagosympathetic

trunk well above the arch of the aorta; their areas of termination correspond exactly to those mentioned for the rabbit, and they also convey fibers for the aortic glomus of the corresponding side.

In the dog we find a somewhat different situation since the aortic fibers upon emerging from the vagosympathetic trunk do not form independent bundles but join other nerves. On the right side they enter the cardiac nerve, which they leave below the subclavia. The aortic fibers coursing within the left cardiac nerve do not emerge from it until it has reached the anterior (ventral) surface of the arch of the aorta. Furthermore, a number of aortic fibers join the slender sympathetic nerve which runs between the innominate and the left subclavia (figs. 1 and 2, cs).

The differences observed in the path followed by the aortic fibers in the dog may account to some extent for the absence of definite epithelioid cell groups above the right subclavia and the arch of the aorta, respectively. However, homologous structures are to be found in a slightly different position. On the right side the group corresponding to the right aortic glomus of the rabbit and cat is represented by a small, poorly defined group of epithelioid cells embedded in the externa of the innominate artery immediately below the root of the right subclavia (fig. 2, ag). Its position is, therefore, essentially similar to that of the right aortic glomus of the guinea pig which occurs below, instead of above, the subclavia (Nonidez, '35; Muratori, '37). A mass of 'paraganglionic' tissue in a similar position is also represented by Boyd ('37, fig. 1) in a 26-mm. human embryo.

If the left cardiac nerve is traced caudad it is seen that at the point where it breaks into branches there is a well-defined group of epithelioid cells (fig. 1, g). This probably is the left aortic glomus, since it receives its blood supply through a small artery (omitted in fig. 1) which springs from the anterior surface of the arch of the aorta. Branches of this artery also reach smaller epithelioid masses located on the anterior surface of the bifurcation of the pulmonary artery near the base

of the ductus arteriosus. A characteristic feature of this artery and its branches is the presence of adventitial nerve arborizations of pressoreceptor type, also noticed in the artery supplying the aortic glomus of the rabbit and cat (Nonidez, '35). Although the sympathetic nerve coursing caudad between the left subclavia and the innominate artery (figs. 1 and 2, cs) carries aortic fibers ending on the arch of the aorta no epithelioid body is present near its termination in the two puppies examined. In the cat and rabbit the left aortic glomus occurs in this location.

The groups of epithelioid cells mentioned above are not the only bodies located in the region of the arch of the aorta. In the space between the latter and the pulmonary there are several cell masses (fig. 2, g', and fig. 6, gl) which are supplied by a small artery leaving the posterior surface of the arch of the aorta (fig. 2, ga). The cell groups under consideration would correspond to what Palme ('34) calls *paraganglion supracardiale superius* in the human. They are present in the two puppies examined, deriving their innervation chiefly from the bundle of thin, deeply stained fibers (fig. 1, pg) which courses in front of the ductus arteriosus after leaving the left cardiac nerve. As stated before, this bundle becomes much flattened upon entering the space between the arch of the aorta and the pulmonary, and it courses in the adventitia of the latter. The bundle soon breaks up into smaller bundles which enter the epithelioid bodies and the ganglia of the plexus located under the arch of the aorta.

A third group of epithelioid bodies is to be found between the ascending aorta and the pulmonary trunk in the vicinity of the left coronary artery, corresponding to Palme's *paraganglion supracardiale inferius* (fig. 2, g''). These bodies receive blood through a branch (omitted in fig. 2) arising from the left coronary artery. In addition there are smaller epithelioid bodies in the plexus of nerve fibers found in the posterior surface of the pulmonary trunk; a larger body occurs between the diverging pulmonary arteries. All these cell groups are lodged in the adventitia of the pulmonary

trunk in close relation to small ganglia scattered in the plexus.

From the preceding description it is evident that the distribution of the epithelioid bodies in the dog closely resembles that found in man. Contrary to expectations, no branch arising from the pulmonary artery for the supply of the epithelioid bodies between the ascending aorta and the pulmonary was found in the two puppies examined. This indicates that the presence of such a branch in kittens is of no fundamental importance. In view of this fact the functional interpretation offered in a previous article (Nonidez, '36) must be abandoned. In a recent note Goormaghtigh and Pannier ('36) claim that the branch of the pulmonary constantly found in cat embryos and kittens becomes obliterated in the adult, and that the epithelioid masses receive arterial blood through an artery springing from the coronary arteries or from the ascending aorta. Further discussion of this interesting observation must await the publication in extenso of the researches of the two workers mentioned. It may be stated, however, that in some cats the occlusion of the branch under consideration fails to take place since it can be injected from the pulmonary artery (Palme, '34; Nonidez, '36).

The structure of the epithelioid bodies is very uniform. The epithelioid cells seem to be of a single type with slightly granular cytoplasm and round or oval nuclei. In some areas they appear closely packed while in others they show a tendency to form irregular strands separated from each other by capillaries. Arterioles are numerous. An interesting feature, already observed in kittens, is very plain in the epithelioid cell groups of the puppy, namely, the absence of clear cut boundaries between the walls of some of the smaller arterial branches and the groups of epithelioid cells. This is illustrated in figure 7, representing a branch (a) of the artery supplying the inferior group of epithelioid bodies (fig. 2, g''). It will be noticed that in the branch entering the cell group *g* the smooth muscle fibers have disappeared and that the epithelioid elements are practically in contact with the

endothelium. This suggests that the epithelioid cells may have been budded off from the arterial wall, or that they represent a transformation of the mesenchyme in its immediate vicinity. The latter interpretation receives support in recent findings by Boyd ('37) on the development of the human carotid glomus and the 'paraganglia' located between the large vessels of the heart.

In regard to the nerve supply of the epithelioid cell groups it may be said that it is very abundant and elaborate, surpassing the innervation of the corresponding structures of the cat and rabbit. The fibers reaching the epithelioid bodies of the dog are for the most part medium sized and small, but a few large fibers also enter the cell groups. With the technique used they are stained black. As they course among the epithelioid elements they break into numerous branches some of which form baskets around the cell bodies. The large thickenings observed in the cat (Nonidez, '36) do not occur in the dog. The finer branches of the arborizations end as minute rings and small club-shaped enlargements closely applied to the cytoplasm of the cells. Ganglion cells scattered among the epithelioid elements were not observed, but small ganglia may be in close contact with the epithelioid bodies, especially with those situated in the nerve plexus of the posterior wall of the pulmonary trunk.

In concluding I should like to state that the structure, mode of innervation and rich blood supply to the epithelioid bodies of the dog suggest once more that we are dealing with complex neurovascular structures intimately associated with the branchial arches of the embryo. The presence of an abundant nerve supply does not necessarily imply a neurogenic origin for the epithelioid cells. In a similar way, the proximity of the epithelioid bodies to the areas of termination of the aortic (depressor) nerve cannot be accepted as evidence indicating that they are closely related to the pressoreceptors. As a matter of fact, in the cat and dog there are also definite pressoreceptor areas in the venae cavae and the pulmonary veins (Nonidez, '37) but no epithelioid bodies appear associated

with them. Ganglia also occur in the vicinity and even in the walls of these vessels—especially in the superior vena cava—but they do not have cell groups suggesting paraganglionic tissue. In the present state of our knowledge all that can be said in regard to the presumed relationship of the pressoreceptor areas and the epithelioid bodies is that the fibers for the latter join the bundle of aortic fibers ending as pressoreceptors; this, however, only applies to the epithelioid bodies near the right subclavia and the arch of the aorta—the aortic glomi—for the fibers to the other epithelioid bodies are conveyed for the most part by the right cardiac nerve, in the dog as well as in the cat and rabbit.

SUMMARY

1. The distribution and paths followed by the aortic (depressor) fibers of the dog are described. On the right side they emerge from the right cardiac nerve (figs. 1 and 2, cn) as a bundle (an) which also carries sympathetic postganglionics and ends on the innominate artery at the base of the right subclavia. On the left side the aortic fibers follow two different paths: a) The majority enter the left cardiac nerve (cn') to end in two pressoreceptor areas, one in the arch of the aorta (a), the other in the distal portion of the pulmonary trunk (a'); b) a smaller number of fibers join a cardio-sympathetic nerve (cs) running caudad between the left subclavia and the innominate artery; they end on the arch of the aorta.

2. The distribution, structure and innervation of the epithelioid bodies ('paraganglia') are considered and discussed. The cell group corresponding to the right aortic glomus of the rabbit and cat is poorly developed and appears embedded in the adventitia of the innominate artery (fig. 2, ag). On the left side a group of epithelioid cells occurs on the anterior surface of the arch of the aorta (fig. 1, g) and it is regarded as corresponding to the left aortic glomus. The other epithelioid bodies appear as two main groups: a) One located under the arch of the aorta, between the latter and the pulmonary (fig. 2, g and fig. 5, gl); b) another (g'') situated

between the ascending aorta and the pulmonary trunk in the vicinity of the left coronary artery. Scattered epithelioid bodies also occur in the plexus of the wall of the pulmonary trunk.

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PLATE 1

EXPLANATION OF FIGURES

All figures in this plate are unretouched photomicrographs taken at the same magnification; 1-day-old puppy. Cajal method.

3 Shows right aortic nerve (an) in transverse section. a, adventitia of the innominate artery; cn, right cardiac nerve. * indicates the point of emergence of the aortic nerve.

4 Transverse section of the left cardiac nerve (figs. 1 and 2, cn'). a, anastomosis of cardiac nerve with left recurrent; p, bundle of preganglionics and fibers to the epithelioid bodies between the arch of the aorta and the pulmonary; s, sympathetic postganglionics. Thick aortic fibers also seen in figure.

5 Transverse section of the cardio-sympathetic nerve (figs. 1 and 2, cs), showing thick and medium-sized aortic fibers and sympathetic postganglionics, s.

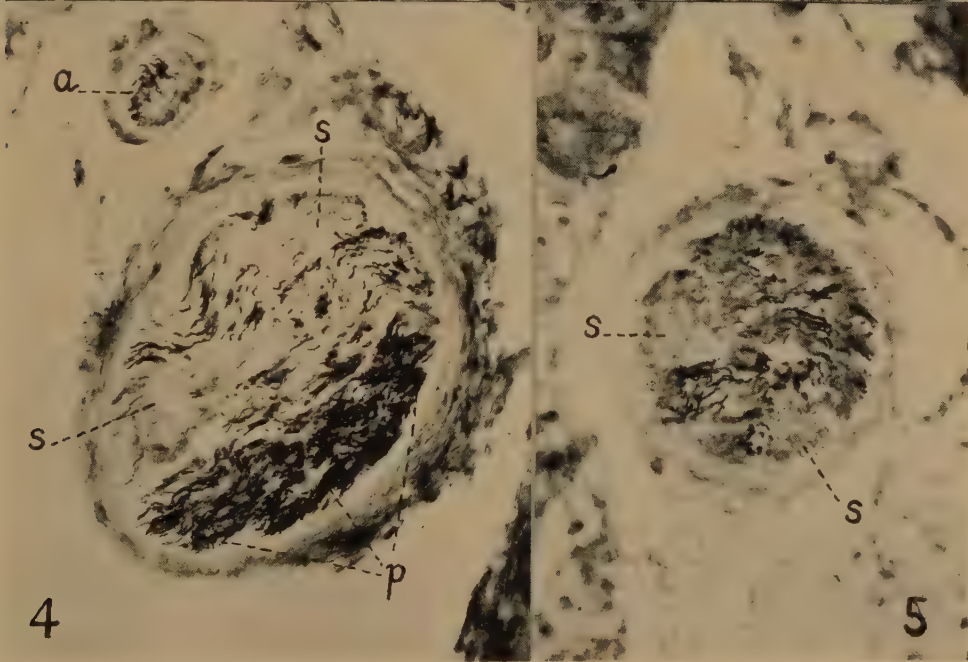
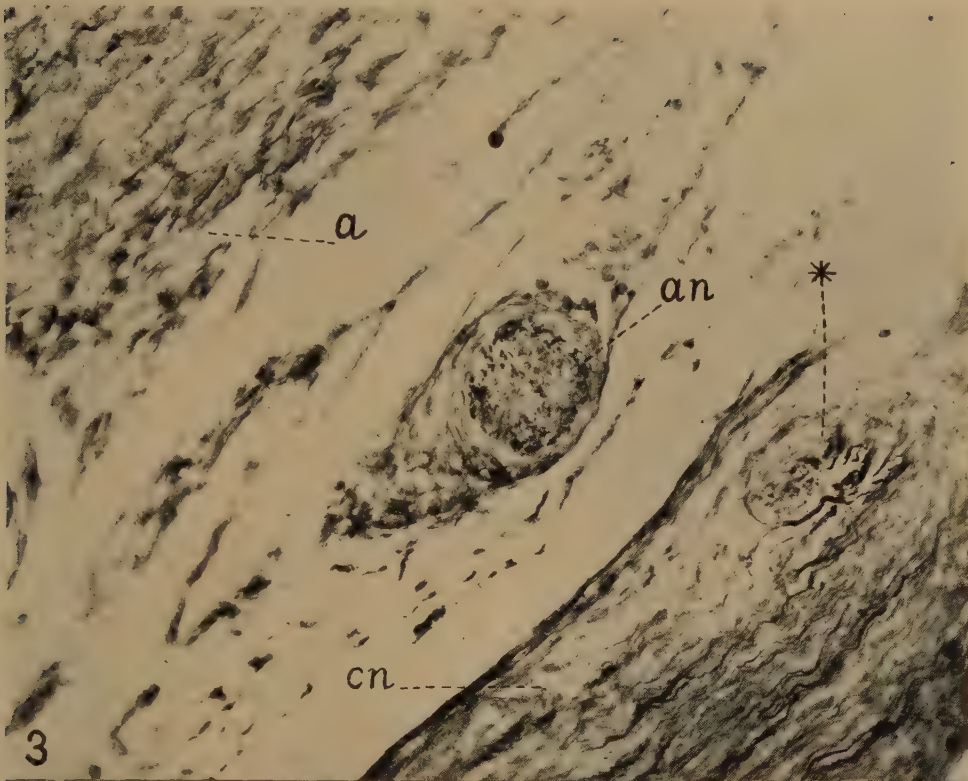
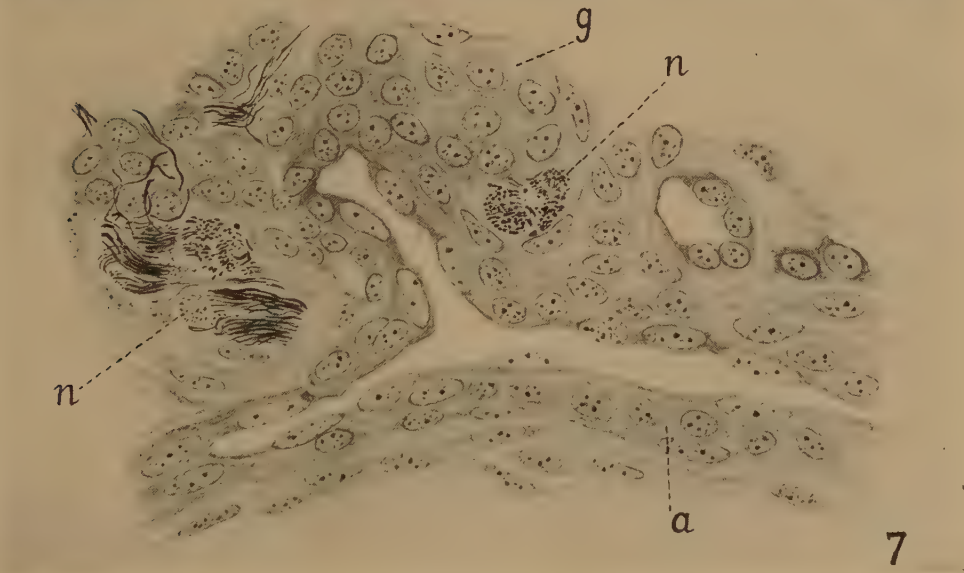
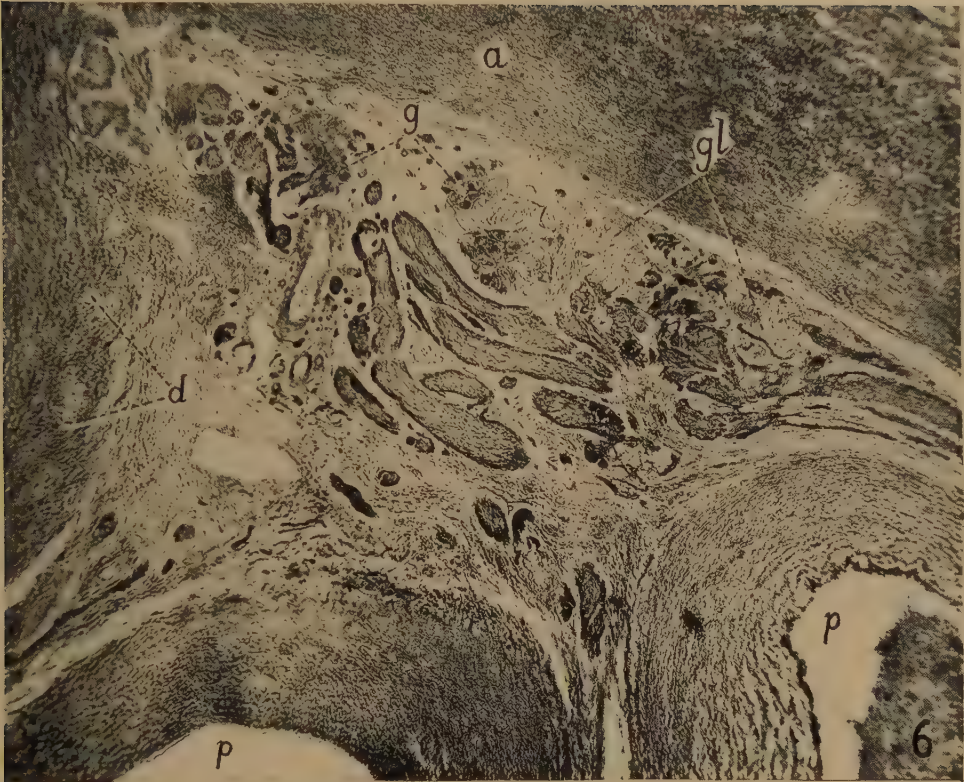


PLATE 2

EXPLANATION OF FIGURES

6 Nerve plexus in the space between the arch of the aorta (a) and the bifurcation of the pulmonary artery (p). d, wall of the ductus arteriosus; g, ganglia of the plexus; gl, epithelioid cell groups (labeled g' in fig. 2). The numerous black dots scattered in the plexus are not precipitates but cross sections of small, deeply stained nerve bundles. From a frontal section of the heart, posterior view.

7 Shows a branch (a) of the artery supplying the inferior group of epithelioid cell masses (fig. 2, g''). g, group of epithelioid cells attached to a branch of the artery; n, nerve bundles.



RELATION OF KIDNEY WEIGHT TO BODY WEIGHT IN THE CAT ¹

V. E. HALL AND W. W. MACGREGOR

Department of Physiology, Stanford University, California

TWO FIGURES

In the course of experiments on kidney function in the cat, we became interested in developing a method for predicting kidney weight from body size in this species. Since the work of Stewart ('21) it has been generally accepted that kidney weight is more nearly proportional to body surface than any other single measure of body size, such as weight or length. This relation has been demonstrated for the rabbit by Taylor, Drury and Addis ('23), for the rat by MacKay and MacKay ('27), for man by MacKay ('32), and is evident in the pooled data of Stewart ('21), Kunkel ('30), Ralli, Brown and Pariente ('31) and MacKay ('32) for the dog. Since body surface is approximately proportional to the 0.67 power of the body weight, the finding of Roaf ('13) that the kidney weight of the cat correlated best with the 1.5 power of the body weight was surprising. The following study was undertaken to determine why the cat appeared to differ so fundamentally from other mammalian species.

METHODS

Fifty male and fifty female cats, varying in size from 110 gm. to 5.04 kg. and in age from a few hours to maturity, were used. The kittens were born in the laboratory, the adults were acquired from dealers. The cats represented a chance mixture

¹Supported in part by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

of the strains constituting the domestic cat population of this vicinity.

The kidneys were excised immediately after death, the capsules removed together with the ureter, vessels and nerves as close to the hilus as possible. No attempt was made to render the kidneys bloodless. Kidney weights are accurate to the centigram; body weights of kittens to the gram and of cats over 1 kilo to 10 gm.

The significance of differences between the means of groups of data in this paper has been calculated by the method of Fisher ('30), and expressed as the chances that such a difference could occur in random sampling.

RESULTS AND DISCUSSION

a. Comparison of left and right kidneys

No evidence was obtained that there is any significant difference between the weights of the right and left kidneys, the small differences occurring being such that in ninety-eight cases out of 100 they would appear in random sampling. This conclusion applies not only to the group as a whole, but also to the adults alone and to the sexes treated separately.

It might be expected that, should one kidney be small, compensatory growth regulating factors might increase the size of the other. If this were the case, the variability of the total kidney weight (the sum of the weights of the two kidneys) ought to be less than that of the left or right kidneys separately.

Such is not the case, for the variability (expressed as the per cent which the standard deviation of the weights is of the mean weight) is practically identical: total kidney weight, 77%; right kidney weight, 76%; left kidney weight, 77%. The same conclusion is supported by the fact that, in any weight group, there is a strong rank correlation between the ratios of right kidney weight to body weight and of left kidney weight to body weight. This would indicate that the regulatory influence governing the amount of renal tissues present under normal conditions acts so perfectly on each kidney that the compensatory mechanism revealed under pathological conditions does not operate. An example of the latter effect

is afforded by the occurrence in one cat of a cyst of the left kidney. Here the total kidney weight of 18.09 gm. was within the limits of normal variation for a cat of that body weight; the parenchymal tissue of the left kidney was reduced to 5.08 gm., while that of the right rose to 13.01 gm.

TABLE 1

Kidney weights,¹ K.W./B.W. ratios and deviations of calculated from observed K.W.

BODY WEIGHT RANGE	NUMBER OF CASES	MEAN K.W.	K.W./B.W. RATIO	MEAN DEVIATIONS	
				Absolute	Relative
Males					
<i>kg.</i>		<i>gm.</i>		<i>gm.</i>	<i>%</i>
0.10-0.30	4	2.65	1.78	0.22	7.51
0.30-0.50	6	5.00	1.23	0.71	17.69
0.50-0.70	7	6.51	1.02	0.51	8.26
0.70-1.50	10	7.41	0.90	4.70	6.74
1.50-3.00	7	21.81	0.80	4.15	19.79
3.00-3.50	9	29.93	0.94	5.00	19.09
3.50-5.10	7	42.97	1.02	6.43	15.70
Females					
0.10-0.30	5	2.23	1.52	0.22	8.88
0.30-0.50	8	4.68	1.26	0.73	20.23
0.50-2.00	6	8.63	0.76	1.56	17.71
2.00-2.50	14	15.39	0.68	2.91	18.96
2.50-3.00	11	19.18	0.72	2.67	12.71
3.00-4.10	6	21.56	0.64	1.85	8.85

¹ 'Kidney weight' here refers to the sum of weights of left and right kidneys.

b. General features of the ratio of K.W. to B.W. in the sexes taken separately

In table 1 are given the kidney weight and the kidney weight-body weight ratios (hereinafter called the K.W./B.W.) of cats grouped according to body weight intervals. The mean values of K.W./B.W. for various body weight ranges are plotted as functions of the body weights in figure 1.

The curve for the male cats lies above that for the females throughout its course. In the range of body weights of 300 to 700 gm. the chances that the excess of the kidney weights of the males over that of the females could occur in random

sampling are three in 100; in the range of 2.83 to 4.02 kg. they are six in 100. In view of the presence of considerable variation in body weight in these ranges, with consequent variation in kidney weights, which would tend to obscure differences from other sources, the differences may be considered valid. A similar sex-difference has been demonstrated to exist in the albino rat by MacKay and MacKay ('27).

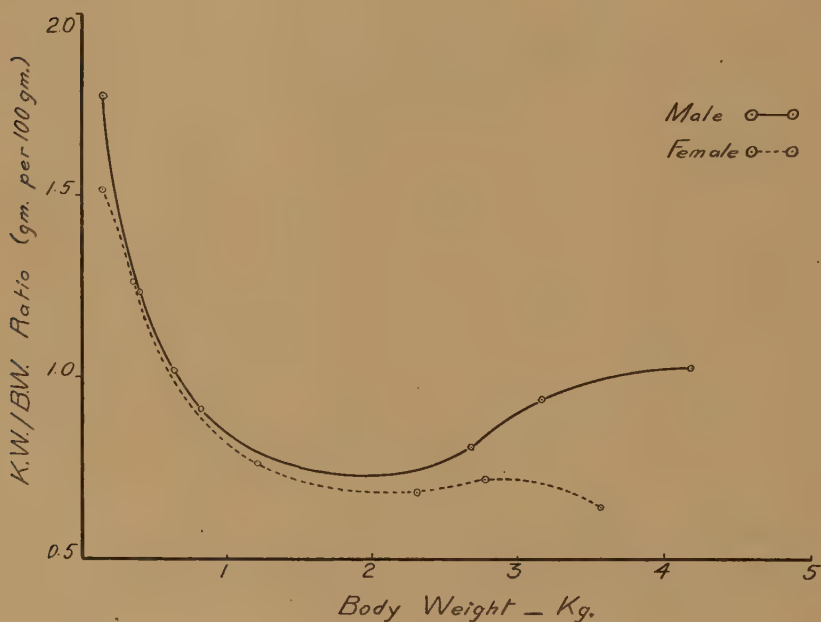


Fig. 1 Relation of relative kidney weight to body weight in the cat.

In both sexes the weight of the kidney relative to the total body weight decreases as the cats grow to a weight of 1.75 kg. This relation agrees in its general features with current conceptions of the relative growth of the thoracic and abdominal viscera (Jackson, '28).

However, the relation no longer holds in the male animals when their body weights exceed 1.75 kg., for here, as the cats' weights increase, their relative kidney weights increase progressively. The chances that the difference between the values

of K.W./B.W. for the body weight ranges of 1.80 to 3.00 kg. and 3.50 to 5.04 kg. could occur in random sampling are six in 100. This peculiarity of the large male cats is precisely the relation of kidney to body weights claimed by Roaf ('13) to be characteristic of the species. Examination of his data shows that all the male cats which he used, the smallest of which weighed 1.35 kg., fell in the size range in which our male cats showed an increasing K.W./B.W. with increasing body weight. Calculation of his data by our method yields results which in general are in agreement with ours. The reason why Roaf's conclusions concerning the kidney to body weight relations in cats appeared to be so radically different from those commonly accepted now becomes evident—he confined his study to a definite body weight range in which, as we have shown, an anomalous situation exists. When the entire range of body weights is considered, the male cats present a picture conforming in broad outline to that currently accepted.

In female animals, our data fail to show the anomalous increase in K.W./B.W. in the higher body weight ranges noted in males. The slight difference in K.W./B.W. for the body weight ranges of 2.00 to 2.60 kg. and 3.00 to 4.50 kg. is one that may occur in half of any such series in random sampling. Roaf's females show a greater mean K.W./B.W. in the body weight range of 2.38 to 3.40 kg. than in that of 1.69 to 2.36 kg. but the difference is one that may occur in one-third of such observations in random sampling.

The female cat may then be concluded to differ from the male in having a relatively constant value for K.W./B.W. in the adults, and in not showing any tendency for this ratio to increase in the heavier individuals.

It is of interest to determine whether the characteristic of large male cats noted above had been previously recognized in other species. In male rabbits, the data of Taylor, Drury and Addis ('23), when treated according to our methods, yield relations of kidney weight to body weight which are very similar to those existing in male cats. The K.W./B.W. for

body weight range of 2.0 to 2.7 kilos is 0.556 gm. per kilogram; that in the range of 2.7 to 3.7 kg. is 0.632 gm. per kilogram. The chances that such a difference could occur in random sampling are five in 1000.

The available data for the dog, published by Stewart ('21), Kunkel ('30), Ralli, Brown and Pariente ('31), and MacKay ('32), have been pooled and treated by our methods. The variability of K.W./B.W. in any weight interval is so great that little can be deduced from the material. However, it is suggestive that the median value of K.W./B.W. for the body weights of 15 to 23 kg. is 3.14 gm. per kilogram while that for the weights of 23 to 40 kg. is 3.63 gm. per kilogram. Since the sex of the dogs is not given in the publications, it has not been possible to separate out the males. Possibly, had this been done, the relation existing in the cat and rabbit might have emerged.

The data on the albino rat gathered by MacKay and MacKay ('27), treated by our method, fails to reveal any tendency for the larger animals of either sex to have relatively heavier kidneys.

No satisfactory interpretation of the newly recognized relations presented above is at hand. Since the study of MacKay and MacKay on the albino rat was the only one made on a single strain of animals, and was also the only one in which the larger males did not have disproportionately large kidneys, it might be suggested that this disproportion was related to the genetic heterogeneity of the cat and rabbit material used by other workers. Thus, it might be suggested that among the strains constituting the populations studied, those reaching the greater final body weights also have relatively larger kidneys. This, however, is contrary to general experience, for among species of the same order, the larger animals have relatively smaller kidneys; thus, while in the dog the total K.W./B.W. has a minimum value of 0.32%, the corresponding value for the cat is 0.64%. We are, therefore, not inclined to credit this genetic suggestion. We are also at a loss to explain why the relation should be shown only among

the male animals. Whatever be the biological significance of this finding, it is clear that it must be taken into consideration in experimental work in which kidney weights are predicted from other biometric data.

c. Exponential relation of kidney weight to body weight

Huxley ('32) has shown that in the growth of many species the relation between the size of a part and the whole body is well represented by the following exponential formula: If K = weight of a part, such as the kidney; W = body weight; and a and b are constants, then

$$K = a W^b, \text{ or} \\ \log K = \log a + b \log W$$

Now, if $\log K$ be plotted on $\log W$, the slope of the line best fitting the points will be b , the required exponent. If the exponent is less than unity, the part is decreasing relative to the whole; if greater, it is increasing.

Applying this procedure to our data relative to kidney and body weights, we have plotted in figure 2, the mean values of $\log K$ on those of $\log W$ for various body weight ranges. The female cats yield points which lie satisfactorily on a straight line the slope of which is 0.71. The points given by the six weight-interval groups of males do not lie on a straight line, but on a curve the slope of which increases progressively with increasing body weight. Although the average slope (best fit of a straight line) is 0.86, the slope for the male kittens (up to 1 kilo) averages 0.64, while that for adult males is about 1.41. Application of our methods to Roaf's data, taking the sexes separately, gave exponents of 1.73 for the male cats and 1.08 for the females, respectively.

Before discussing the significance of these findings, we may examine the exponents which we have calculated from the published data on other species. The relations presented by our male cats are almost exactly duplicated in the male rabbits studied by Taylor, Drury and Addis ('23). The exponent for body weights up to 1.41 kg. is 0.42; for 1.41 to

2.51 kg., 1.00; and for 2.51 to 3.69 kg., 1.41. In the dog, the pooled data referred to in the preceding section yield a value of approximately 0.7 over the whole body weight range, while in the albino rat the data of MacKay and MacKay ('27) give 0.595 for the males and 0.555 for the females, again throughout the entire range of body size studied.

It thus appears that the precise relation between kidney weight and body weight varies considerable between species

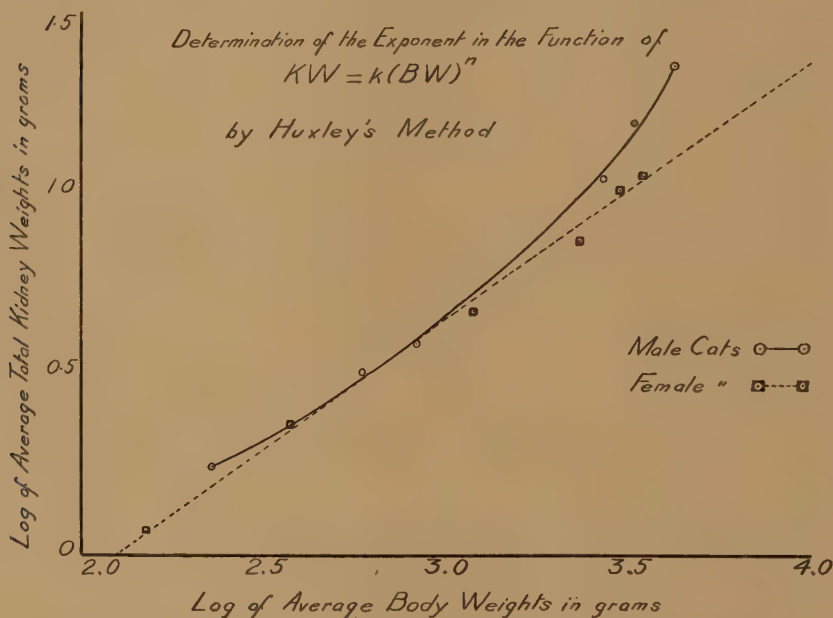


Fig. 2 Logarithmic relations of kidney and body weights in the cat.

and between the sexes in those species in which sexes have been studied separately. Moreover, in male cats and rabbits it varies with the range of body sizes under consideration. That such variation would occur might well be expected since the relation of any part to the whole body is affected by the relative development of all other portions. The general theory of such relations as a problem of body form is well formulated by Huxley ('32). One aspect of the relations merits special mention: in all the cases we have been able to examine, the

exponent for males is larger than that for females of corresponding size. While this may be an expression of the greater quantity of adipose tissue in the females, it may also be related to sex differences in metabolic processes. In the cat, the quantity of intracellular fat in the kidney tubules of the male is markedly greater than in that of the non-pregnant female, according to unpublished observations by D. Rytand and one of us.

The bearing of these findings upon current concepts of the relation of kidney weight to body size may now be considered. Since the body surface is proportional to the two-thirds power of the body weight, any part which is strictly proportional to the body surface should yield when treated by the above methods an exponent of 0.67. In no instance examined above was this precisely the case. Consequently, in no case is the kidney weight strictly proportional to the body surface. It is true that in our female cats, and in dogs and rats, the difference between the calculated exponent and 0.67 is not great, but, as will be shown in the next section, the discrepancy is great enough to influence distinctly the accuracy of prediction of kidney weight from body weight. However, in the male cats and rabbits, the exponents not merely fail to approximate the 0.67 of conventional concepts, but surpass unity, reaching in our male cats 1.41. These exponents express in another manner the relation which has been described earlier in this paper—that the kidney weight of large cats increases disproportionately with increasing body weight. The relation to body surface in these animals now becomes still more anomalous. Thus, if the body surface is proportional to the 0.67 power of the body weight, while the kidney weight is proportional to the 1.41 power, then the kidney weight must be proportional to the $\frac{1.41}{0.67}$ or 2.10 power of the body surface, or approximately to its square.

We conclude that the generalization so prevalent in the literature that the kidney weight is proportional to the body surface is only roughly valid. While it is often a more accurate relation than that to the body weight as such, still

more accurate relations may be formulated. The fact that these new relations may involve such mathematical abstractions as fractional exponential relations, instead of readily grasped bodily measures such as the surface area, is no disadvantage. In much work in this field such abstractions are actually employed, under the guise of calculating the body surface. Thus, MacKay and MacKay ('27) employed the Meeh formula for their rats' surface areas, while MacKay ('32) used the DuBois method based on the body length and weight for man. When the quantitative relation between the body and one of its parts is under consideration, nothing is gained statistically and much may be lost in the attempt to

TABLE 2
Relative value of kidney weight prediction methods (mean absolute and per cent errors). Female cats

	BODY WEIGHT RANGE	EXPONENTIAL METHODS				GROUP RATIO K.W./B.W.	
		0.67		0.71			
		Absolute	Per cent	Absolute	Per cent	Absolute	Per cent
	<i>kg.</i>	<i>gm.</i>		<i>gm.</i>		<i>gm.</i>	
Whole group		2.34	26.37	2.11	18.45	1.90	15.59
Upper third	4.02-2.60	2.66	11.55	2.53	11.58	2.48	11.57
Middle third	2.55-1.50	2.65	18.58	2.97	22.02	2.52	18.01
Lower third	0.97-0.10	1.68	50.32	0.77	21.96	0.64	17.29

simplify interpretation by the introduction of some intermediate measure. It is preferable to employ mathematical relations sufficiently complex to represent the complexities actually presented by our material.

d. Relative value of prediction methods for cat kidney weight

In table 2 are given the results of the prediction of the kidney weight from the body weight by various methods as applied to our female cats. The first is that based on the 0.67 power of the body weight, a method equivalent to basing the calculation on the body surface when the latter is estimated from the body weight by the Meeh formula. The prediction formula is:

$$\text{K.W.} = 10.5 (\text{B.W.})^{0.67} - 2.62$$

This formula was obtained by dividing all the cases into two equal groups, one consisting of the larger, the other of the smaller, cats, and obtaining for each group the mean values of K.W. and of (B.W.)^{0.67}, from which the regression equation was calculated.

The second method consisted in using the exponent of 0.71, that derived in the previous section, to calculate the regression equation, which was :

$$\text{K.W.} = 9.825 (\text{B.W.})^{0.71} - 1.09$$

Finally, the third method employed depended upon the use of the average ratio of K.W./B.W. in each of six body weight ranges. Here the body weight has been multiplied by the average ratio and the resulting product held to represent the kidney weight.

In evaluating the three methods, we have calculated for each cat the kidney weight as predicted by each of the three methods, and determined the absolute and percentage deviation of each predicted weight from the observed kidney weight. The table gives the deviations for the whole group, and for the upper, middle and lower thirds separately. The two methods employing exponential relations are about equally good in their prediction of the kidney weights of middle sized and large cats, but those of kittens are much better predicted by the exponent of 0.71. The kidney weights of the kittens are somewhat under estimated by both methods but much more so by that based on the 0.67 exponent. Thus, in a kitten of 215 gm. body weight, the kidneys actually weighed 2.97 gm.; the method using an exponent of 0.67 predicted 1.13 gm., that using 0.71, 2.21 gm., the per cent errors being 61.9 and 25.6, respectively. The superiority of the exponent of 0.71 in the lower body weight range is almost the entire reason for the fact that the mean per cent deviation of predicted from observed kidney weight calculated from this exponent is 18.45, while that calculated from the exponent of 0.67 is 26.37.

The existence of a systematic underestimation of the kidney weights of the kittens by both methods implies the absence of

a truly linear relation between $\log (K.W.)$ and $(B.W.)$. The simplest method of avoiding this difficulty appeared to be that of dividing the animals into six body weight ranges, and of treating each such range separately as described above. Since the direction of the deviations showed no consistent tendency to be opposite in the heavier and lighter cats in any group, no error is introduced in using the $K.W./B.W.$ ratio instead of a formula with the exponent of $B.W.$ proper to that body weight range. The improvement over the exponential methods is definite but not large, the overall average deviation becoming 15.04%. This third method has the double advantage of being slightly more accurate than the exponential method employing the best possible value, 0.71, and of being much simpler to employ in practical use. We therefore recommend it for the prediction of kidney weight from body weight in this species. Table 1 contains the data necessary for the use of the recommended procedure.

Since the exponent in male cats varies progressively through the body weight range, it is obviously not rational to base a prediction method on it. The separate treatment of restricted body weight ranges has been employed, as for the female animals. The necessary data will also be found in table 1.

SUMMARY

1. In female cats, the kidney weight relative to the body weight declines with increasing body weight throughout the entire body weight range. The kidney weight is more nearly proportional to the 0.71 power of the body weight than to the 0.67 power.

2. In male cats, the kidney weight-body weight ratio, highest in newborn kittens, declines to a minimum in young adults, then rises again in the largest animals. The exponential relation is not constant throughout the body weight range, increasing progressively from 0.64 in kittens to 1.41 for the largest cats.

3. On the basis of these findings on cats and those on other species reported in the literature, it is concluded that the prevailing concept that the kidney weight is proportional to the body surface is only approximately valid, and that, in certain cases, may be quite erroneous. Other relations of superior accuracy may be formulated.

4. A method for predicting kidney weight from body weight for experimental use in the cat is presented.

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A RAPID METHOD OF PREPARING GROUND TOOTH SECTIONS

GERRIT BEVELANDER

Department of Anatomy, New York University, College of Dentistry

ONE PLATE (FOUR FIGURES)

INTRODUCTION

To the uninitiated, the task of preparing ground sections of teeth suitable for microscopic study seems beset with insuperable difficulties. For this reason it is felt that many potential investigators of tooth structure are more or less permanently discouraged from undertaking such studies. One needs only to follow the method advocated by Black ('29), to lose any enthusiasm which one may have acquired for this type of investigation. Galigher ('34), in his excellent text on microscopic technique is even more discouraging, for he states that the preparation of complete ground sections of teeth is extremely difficult and the beginner is advised not to try. The net effect of such conditions is one which is hardly conducive to the attempted solution of a problem dealing with the histology or pathology of teeth. One is left with the impression that the technical difficulties are practically unmountable.

In presenting a description of the following method, it is hoped that some students interested in the anatomy of teeth who have heretofore despaired of surmounting the technical difficulties offered by their problem, will resume their labors when they have given this method a trial.

In what follows, an outline is presented which can readily be modified or adapted to any particular need. This method offers a simple, direct and rapid means of preparing sections

free from all but the most simple mechanical or technical procedures. Throughout we have borne in mind that the teeth are biological structures and that it is necessary to handle them with due regard to this fact.

It has been felt that the prevailing method of mounting teeth on a slide in balsam, or variations of this method, as the initial step in preparing sections is a time wasting procedure. Frequently the specimen becomes detached on subsequent handling. Furthermore, following such treatment, the specimen is usually dry and brittle, with the result that the final preparation contains many unnecessary fractures. Another method in common use is to place a tooth in a vise and remove a section with a saw. This method has many disadvantages as those who have had occasion to make this type of preparation will realize.

In the present method the preparation of tooth sections has been reduced to three operations consuming very little time.

APPARATUS

The materials necessary for grinding tooth sections according to this method are as follows: Two small blocks of wood which are designated the tooth carrier. These blocks are 5 inches long, $\frac{3}{4}$ inch wide, and $\frac{3}{8}$ inch thick. Each block is tapered, and in the tapered ends a slot is cut after which the two blocks are held in place by means of a wing-top clamp. The detail of this arrangement is shown in figures 3 and 4.

We also find the following apparatus convenient although not absolutely necessary. As shown in figure 2 this device is a highly simplified bench table. A piece of $\frac{3}{4}$ inch straight planking 1 foot long is cut so as to present an inclined surface about 4 inches at the top of the incline and 3 inches at the lower end. (These dimensions are for use with a Ritter dental lathe.) The inclined surface is lapped on both sides to provide a guide for the tooth carrier (fig. 1). As shown in figure 2, this apparatus is held in place by means of a base board clamped to a table. A light coat of paraffin applied to the trough of the inclined surface serves as a lubricant for

the carrier which is dimensioned to fit snugly into the trough and yet be freely movable. The other requisites are: a motor fitted with a chuck, a mandril, carborundum separating disks 1 inch in diameter, a dropping device for wetting the specimen, and a shield to prevent spattering. With the apparatus listed above, it is possible to cut a tooth slab 0.5 to 1.0 mm. thick in one operation which consumes less than 5 minutes.

For the final grinding a glass wheel $3\frac{1}{2}$ inches in diameter is used, which is drilled in the center so that it can be mounted on the motor. The wheel may be either smooth or slightly roughened. A few small blocks of wood 1 inch in greatest dimension, and some absorbent cotton complete the requirements for this step. The mounting process requires only reagents commonly found in any technique laboratory so they will not be enumerated here.

METHOD

1. *Treatment of specimens to be sectioned.* Freshly extracted teeth are placed in 5% saline-formol solution for an hour or as much time as is necessary. This treatment fixes the tissue, keeps it moist and also serves as a bacteriocidal agent.

Orient and mount the tooth in the carrier and clamp it firmly between its jaws.¹ The tooth is now ready to be sectioned either by guiding with the hands, or by placing the carrier in the trough of the inclined plane. Bring the free edge of the tooth in contact with the separating disk previously mounted in a chuck. Make any adjustments of carrier or plane that are necessary, turn on a copious flow of water, start the motor and gently push the tooth against the edge of the disk. If the tooth is to be cut in half use one disk, if a thin slab is desired use two disks. The thickness of the resulting slabs will of course depend upon the distance which separates the two disks. A few trial preparations will show the operator how to adjust the apparatus to suit his particular needs. Several sections of the same or different teeth may be

¹ The dimensions suggested for the tooth carrier are designed for human teeth. Any or all of this apparatus may be adapted or modified to meet individual needs.

prepared in a short time and stored in containers of saline-formol for an indefinite period.

2. *Final grinding and polishing.* The tooth slab is cut down to its final thickness as follows: Cover one surface of a small wooden block previously mentioned with a thin layer of absorbent cotton. Thoroughly moisten the cotton and press the tooth slab firmly into the cotton spread. It has been our experience that in the later grinding operations the tooth slab adheres firmly to the cotton spread, so that in effect this preparation makes a very efficient holder for the sections in the final grinding and polishing operations. During the process of cutting and polishing, the wheel must be kept flooded with water to prevent heat from friction and also to keep the disk free from tooth particles which if left to accumulate on the disk will scratch the preparation. The slab is next brought in contact with the disk, held firmly in place, and the disk is rotated. By varying the pressure of the slab against the cutting surface different effects can be obtained, a light pressure will usually polish, heavy pressure will result in rapid cutting.

The slab is held in contact with the rotating disk until upon removal and examination it is noted that the section is free from irregularities and has a high polish. It is then removed from the block, reversed and the same procedure as outlined above is repeated. The approximate thickness of the section can be ascertained either by direct examination or by observing the color of the slab through the opposite side of the glass wheel. When it loses its opaque character it must be handled carefully for it then approaches the density required for microscopic examination. Tooth slabs can be cut to a thickness suitable for sections in the relatively short time of from 10 to 20 minutes.

3. *Final step.* After the section has reached the desired thickness, it is washed in water and examined under the microscope for detail and scratches. If the surface is scratched, it is rubbed on a strop to apply the final polish although the glass wheel usually achieves this sufficiently well. In the next

step the section is passed through the alcohols and thoroughly dried between two pieces of blotting paper.

In mounting the section a copper warming plate and a moderate gas flame is used. A small amount of Canada balsam is heated on the plate until it solidifies on coming in contact with air. Following this, a drop of balsam is placed on a clean slide and cover glass. The section is now affixed to the drop of balsam on the slide and the cover glass inverted over it. If the preparation does not flatten sufficiently, it is heated moderately on the plate until the balsam just begins to run. With the rubber end of a pencil the cover glass is gently pressed in place. When this is done the slide is plunged into a dish of cold water. After a few minutes the balsam will be sufficiently hard to trim away any excess. The slide is now ready for use.

SUMMARY

1. A direct method of preparing ground sections of teeth suitable for microscopic study has been described.

2. This method outlines the procedures necessary for handling the tooth from the time of extraction until it is mounted on the slide and in addition provides a detailed description of the apparatus necessary to follow this outline.

3. The advantages of this method are: 1) its simplicity; 2) the elimination of all but a few simple mechanical appliances; and 3) the great speed with which sections can be made available for study.

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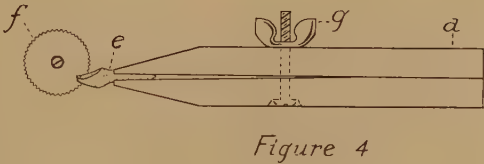
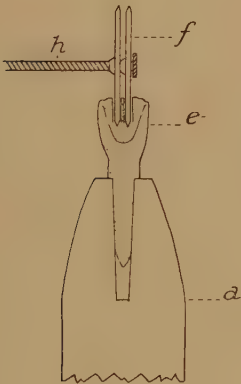
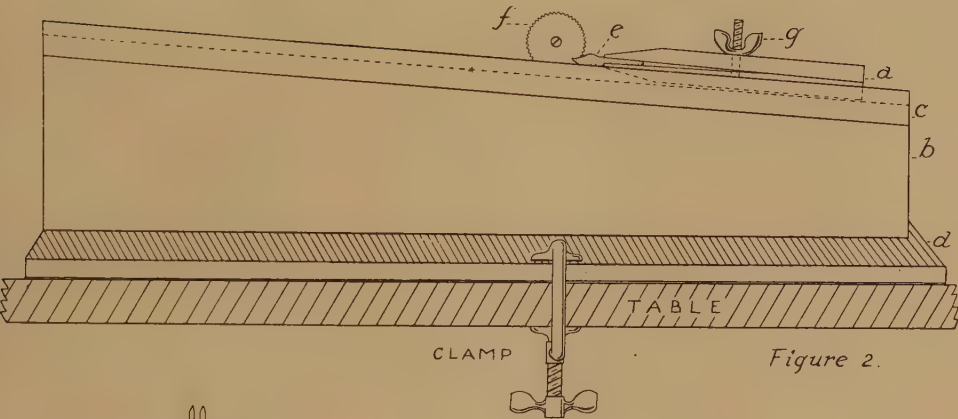
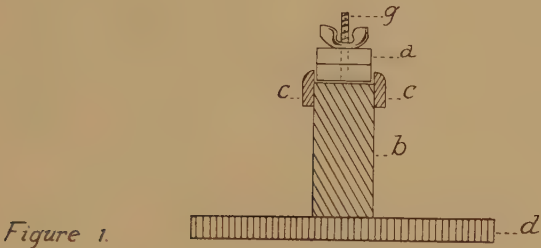
PLATE 1

EXPLANATION OF FIGURES

- 1 End view of inclined plane showing carrier in groove.
- 2 Side view of plane, carrier, tooth and clamp all in position as used in cutting operation.
- 3 Detail of slotted end of tooth carrier showing how tooth is held in place during removal of slab.
- 4 Detailed side view of carrier, with tooth clamped in place showing position of separating disk during cutting operation.

a, tooth carrier
b, inclined plane
c, lap strips
d, base board

e, tooth
f, separating disk
g, clamp
h, mandril



ON THE FAT DISTRIBUTION IN MITOCHONDRIA OF THE GUINEA PIG LIVER ¹

R. R. BENSLEY

Department of Anatomy, The University of Chicago

In 1934 Bensley and Hoerr succeeded in separating for analysis the mitochondria of the hepatic cell of the guinea pig. They found that the mitochondria consisted of protein and fat in the approximate ratio of 6 to 4, and that the fatty substance was devoid of acetone-insoluble components, lecithin and cephalin. They also found that the mitochondria so separated readily underwent changes in solubility.

It is a well-established fact that mitochondria are highly labile structures. They disappear rapidly from the cell post-mortem. Even in good mitochondrial fixatives they are well fixed only at the surface of the block of material. They disappear from material fixed in the organic solvents (alcohol, acetone, chloroform and ether) and in fixing fluids containing a high content of acetic acid. Raising the tissue to a temperature of 48 to 50° also results in disappearance of the mitochondria as morphologic units. These properties have naturally been interpreted as solubilities or melting point, as the case may be.

Bensley and Gersh ('33), by the use of the freezing-drying method, found, on the contrary, that the mitochondria of the hepatic cell of *Amblystoma* in the dried form were not soluble, nor were they visibly altered by the treatment with the reagents mentioned above. After extraction in the Soxhlet apparatus with alcohol, chloroform, ether, acetone, or petroleum ether, no change was visible. Moreover, acetic acid did not dissolve them from the sections of frozen-dried material, nor did high temperatures result in their disappearance.

¹ This research was aided by a grant to The University of Chicago by the Rockefeller Foundation.

There is here an obvious contradiction in the observed facts. Whether this contradiction is a real one, or due to misinterpretations of the facts, can only be determined by increase in our knowledge of the chemical nature of the mitochondria themselves. We have been prone to interpret as solubilities the disappearance of morphological constituents after application of a solvent. It is equally attractive to interpret as melting point the disappearance of a morphological constituent due to rising temperature. There is, however, another possibility in both instances that has received little consideration, namely, that the structure in question has disappeared from view not because its constituents have been dissolved by the reagents or melted, but because they have been dispersed in the protoplasm and no longer form an aggregate.

It is obviously necessary to know more about the proteins and fats found in separated and dried mitochondria by Bensley and Hoerr. For that reason I have devoted a considerable amount of effort to the preparation and analysis of the mitochondrial fats.

The preparations of mitochondria have been made by the method described in the paper of Bensley and Hoerr. It must be emphasized that these efforts do not always result in successful preparations but that microscopical checks of the preparations are necessary at every stage of the process. Large contaminations of the mitochondrial preparations may occur and be unrecognized without the microscopical check, but will be indicated on analyses by increase in the lecithin content, in the percentage of solid fats, and in the lecithin/cholesterol ratio.

In separating the mitochondria from the emulsion of the hepatic cells by centrifugation, the color of the mitochondria is of some aid. They are quite distinctly yellowish. If one depends, however, solely upon the difference in color for identification of the mitochondrial portion of the precipitation, he will recover apparently a much larger total mass of mitochondria but the product will be highly contaminated with other cell products. The reason is that in the primary emulsion

there are cell fragments containing mitochondria which precipitate by centrifuging just ahead of the free mitochondria but do not form a separate layer distinguishable from mitochondria in color, because the mitochondria present in these cell fragments confer their own color upon the mass.

One of the great difficulties in preparing the mitochondria free from other products was the tendency for fragments of cytoplasm insoluble in the salt solution used for the suspension, to float with the mitochondria. These, however, can be largely removed by long-continued centrifugation at very low speed in the first globulin-rich emulsion. They cannot be separated once the globulin solution has been replaced by salt solutions. Even where the best separation of the mitochondria is obtained, there still is a small amount of contamination due to the adherence of a small film of insoluble cytoplasmic protein to the surface of the particle.

The use of isotonic solutions of sucrose or of sodium citrate for washing the blood out of the liver is contraindicated, because in these instances coacervation takes place in the globulin solution and minute particles, difficult to separate from the mitochondria, make their appearance.

As a final stage in the preparation of mitochondria, Bensley and Hoerr suspended them in slightly acidulated water and then centrifuged them. At this stage, if the preparation is contaminated, a jelly-like transparent mass will separate out at the extreme bottom of the centrifuge tube. By using conical tubes, even such a preparation may be saved because the tip of the mass after drying can be rejected, but such preparations also will show higher lecithin content, indicating some contamination of the upper portions of the cake. It is better to separate the contaminating cell substances as early as possible.

To obtain the total fats, the mitochondrial preparation is weighed into the extraction capsule of the Soxhlet apparatus and extracted for 48 hours continuously with hot alcohol; this also extracts any sodium chloride which is present in the preparation. The preparation is further extracted for 24 hours in the same apparatus with ether; and finally for a similar

period with chloroform. The extracts are combined, evaporated to dryness on a water bath under nitrogen, re-dissolved in chloroform, and filtered to get rid of the salts. The chloroform solution is evaporated to dryness under nitrogen and weighed.

Lecithin was determined by dissolving in a small quantity of ether and precipitating in acetone. This operation was repeated three times to complete the separation from the acetone-soluble fats, and the product dried and weighed. Since the amount available for analysis was small, and since I lacked the necessary apparatus to utilize the oxidation method of Bloor ('29) or those of Kirk, Page and van Slyke ('34), the addition of magnesium chloride to assist in the precipitation of the lecithin was avoided.

The acetone-soluble fats were then saponified and the non-saponifiable portion separated in the usual way by petroleum ether. The non-saponifiables recovered from the petroleum ether fraction in the earlier analyses were determined by weight, but since the non-saponifiables contain a small quantity of unknown material, cholesterol was determined in the later ones by the more satisfactory digitonin method of Windaus ('10). From the soap fraction, the fatty acids were recovered and weighed. In some instances the fatty acids were separated into saturated and unsaturated fractions by the lead soap method of Varrentrapp (see Leathes and Raper, 'The Fats,' p. 82) or Twitchell (1895), and the iodine number determined by the method of Wijs (see Leathes and Raper, 'The Fats,' p. 70).

The work has been much handicapped by inexperience and by lack of appropriate chemical apparatus. The separation of the fats is an art which cannot be acquired by reading and meditation. For that reason, the later analyses are probably the more trustworthy. The results, however, of these analyses are presented just as they were obtained and in their chronological order, and the comments which accompany the table will indicate the writer's opinion of the quality of the preparation and the validity of the results.

In this series nos. 5, 8, 10 and 12 are obviously specimens highly contaminated by other cell products as indicated by the high content of lecithin, and in the case of no. 12 by the high lecithin/cholesterol ratio. The cholesterol determinations in nos. 6 and 7 were made by weighing the non-saponifiables after saponification, and the high content of cholesterol in no. 7 is obviously due to incomplete saponification. If we ignore these criticisms, the percentage composition of dried mitochondria

TABLE 1
Quantitative data of analyses of mitochondria

PREPARATION NO.	MITOCHONDRIA	FATS	FATS	ACETONE INSOLUBLE FATS	ACETONE INSOLUBLE FATS	CHOLESTEROL	CHOLESTEROL
	gm.	gm.	%	gm.	%	gm.	%
1	0.0770	0.0322	41.4	0.0028	3.63		
2	0.1162	0.0514	44.2	None			
3	0.0665	0.0292	43.9	None			
4	0.2117	0.0713	33.7	None			
5	0.1302	0.0405	30.9	0.0074	5.68		
6	0.2017	0.0640	31.7	0.0057	2.80	0.0046	2.28
7	0.1737	0.0512	29.4	0.0067	3.79	0.0082	4.7
8	0.1523	0.0514	33.7	0.0190	12.4		
9	0.0541	0.0198	36.6	0.0026	4.8	0.0007	1.29
10	0.0667	0.0296	44.3	0.0060	8.9		
11	0.0722	0.0301	41.69	0.0025	3.44		
12	0.1640	0.0672	40.9	0.0111	6.7	0.00155	0.94
13	0.0840	0.0300	35.7	0.0037	4.4		
14	0.1626	0.0624	38.3	0.0056	3.44	0.0028	1.72
15	0.1532	0.0485	31.6	0.0058	3.77	0.0015	0.98
16	0.2038	0.0592	29.0	0.0088	4.31	0.00515	2.52

of the guinea pig liver derived by summation of all these results is as follows:

Proteins and unknowns	64.67
Glycerides	28.88
Lecithin, cephalin, etc.	4.2
Cholesterol	2.25

The cholesterol percentage in this computation is obtained by summation of the totals in the seven analyses in which the cholesterol was determined quantitatively. In all samples, cholesterol was positive to the Liebermann-Burchard test. The

table shows a highly variable fat content, ranging from 29.0 to 44.3%. Some of this variability is doubtless due to varying quantities of sodium chloride retained in the preparation, but there is also doubtless a considerable variability in the fat content, functional in source.

With such small quantities of cholesterol recovered, some doubt naturally enters one's mind as to whether it is derived from the mitochondria themselves or from contamination with other cell products. A comparison of the lecithin/cholesterol ratios, however, answers this question. In the whole liver the ratio of lecithin to cholesterol is approximately 12:1, and in the fractions of the liver other than mitochondria, obtained by chemical separation, the ratio does not fall below 6:1, except in the 10% sodium chloride extract. This indicates that the cholesterol is unquestionably a component of the mitochondria, although probably in lower proportions than indicated in the table above.

The question as to whether lecithin is a component of the mitochondria, or present only as a result of contamination of the preparation with other cell products, cannot be answered definitely. Preparations 2, 3 and 4, in which no lecithin was discovered, were made early in the work when I had not yet learned that a slight opalescence on addition of acetone not separable by prolonged centrifugation could be resolved into a definite precipitate by evaporating the preparation to dryness and proceeding more cautiously to solution and precipitation. For this reason, I think that the zero lecithins of 2, 3 and 4 are erroneous, but I am still in doubt as to whether the mitochondria contain any lecithin at all since all preparations have some contamination with other cell products and all the lecithin found in the preparation may be from this source.

The high content of fat in mitochondria found in these analyses will support the conviction that many investigators have had as to the lipoidal nature of mitochondria. But it must be borne in mind that the predominating fats of these structures are glycerides, not phospholipins. Moreover, one

asks himself whether the high content of fat in the mitochondria distinguishes it from the other components of the protoplasm of the liver cell? The answer to this question can only be obtained by comparing the composition of the mitochondria with the composition of the cell as a whole.

The facts included in table 2 are obtained from analyses of the whole liver and from analyses of fractions obtained by the method employed by Plosz (1873). In the case of whole liver, the cholesterol percentage is taken from the work of Mayer and Schaeffer ('13). The mixed globulins were obtained by extracting the liver cells and their fragments with 0.85% salt solution. The solution so obtained was cleared by

TABLE 2
Comparative fat contents of different preparations from guinea pig liver

PREPARATION	PROTEINS AND UNKNOWN	TOTAL FATS	LECITHIN	CHO- LESTEROL	GLYCERIDES	LECITHIN CHOLESTEROL
	%	%	%	%	%	ratio
Dried liver	83.4	16.6	7.4	0.60	8.6	12.3
Mixed globulins	86.6	13.4	5.9	0.59	6.91	10
Plasmosin	74.2	25.8	1.16	0.46	24.18	2.5
Cell residue	59.61	40.39	12.76	1.93	25.7	6.58
Mitochondria	64.67	35.3	4.2	2.25	28.88	1.86

centrifugation slightly acidified, flocculated by heating to 74°C., and the precipitate collected, washed, and dried.

Plosz (loc. cit.) found that after such extraction a second yield of protein substances could be obtained by extraction with 10% solution of sodium chloride. The substance so extracted is recovered by acidification with acetic acid or simply by dilution with water. This substance was first studied by Miescher in 1871, who called it the hyalin substance of Rovida. It is a substance of such extraordinary properties that a further study of it promises interesting results. Because of its resemblance in general properties to myosin, I suggest it be called, for the sake of brevity, plasmosin. Plosz (1873) and Haliburton (1892) were impressed by this resemblance, but failed to find any antecedent in the liver cell

by the methods usually employed for the separation of myosinogen.

The mixed globulins and the plasmosin both carry a high content of fat, although they have been recovered from optically-clear solutions. The analyses given in table 2 are analyses of preparations and not averages. The cell residue was recovered by centrifugation and washing and drying after successive extractions with 0.85% sodium chloride, 10% sodium chloride, and N/200 ammonia.

It may be gathered from inspection of this table that large quantities of fatty substances may be carried by solutions that are optically clear. This fact is fully appreciated by experienced fat chemists, as, for example, see Leathes and Raper ('The Fats,' p. 213) where the authors say, "These fluids, serum at any rate, may contain in the case of the eel, 26 grms. of fatty substances in the litre, including 6 grms. of cholesterol, all of which is invisible in a clear fluid"; or, A. P. Mathews (Physiological Chemistry, p. 108), "In the author's opinion there is no satisfactory evidence as yet of the existence of any such particularly lipin-like outer membrane and experiments indicate that the lipin character of the cytoplasm extends throughout it." Anatomists, on the contrary, have always had great confidence in their ability to see fats if they were present, a fact which probably explains the persistent search for the ever-present lipoids in such structures as mitochondria, Golgi apparatus, dictyosomes, secretion granules and what-not.

An inspection of table 2 reveals that mitochondria do not stand alone as the exclusive bearers of the masked fats in the cell, and that they differ in this respect from the other portions of the cell only in the proportion of fats to proteins and in the kind of fats which the mitochondria harbor. In the mitochondrial fats the simple glycerides of fatty acids predominate and lecithin is small in amount, while in the other cell components, with the exception of plasmosin, lecithin forms a large percentage of the total fat extracted. In the case of plasmosin the low lecithin percentage is accounted for

by the fact that 10% salt solution precipitates lecithin from its sols, and so the lecithin remains in the cell residue.

The fat of mitochondria in the liver cell of the guinea pig differs from the general masked fat of the protoplasm in its high degree of unsaturation. This is apparent if one compares the fat extracted from mitochondria and the fat extracted from whole liver; the former is a slightly yellowish oil which remains liquid at room temperature, while, under the same condition, the fat extracted from whole liver is a yellowish solid. This statement is true only for the guinea pig liver, because the free fats of the cat's liver are also highly unsaturated fats.

The fatty acids from mitochondria when separated also constitute a yellowish oil, liquid at ordinary temperatures. In preparation no. 14, I recovered from the saponification 0.0437 gm. of fatty acids. By the lead soap method I separated from this 0.0284 gm. of unsaturated fatty acids which absorbed 0.04039 gm. iodine. Its iodine number thus is 142.2, equivalent to a mixture of 42.2% of oleic acid and 57.8% linoleic acid. Of course there is no assurance that these acids are present since arachidonic, an acid of much higher degree of unsaturation, has been separated from mammalian liver by Hartley ('09) and Bloor ('29).

We have now to consider this extraordinary case of a morphological component of the cell which contains a high percentage of fat, highly unsaturated and yet does not blacken with osmic acid nor stain with Sudan III. While occasionally one may find discrete fat droplets inside of the mitochondrial filaments or granules, it is by no means a common occurrence. So we may presume that the fat of the mitochondria is not discrete but is dispersed in an ultramicroscopic form. Since the mitochondria give a Millon reaction which is equally intense in all parts of the filament or granule, it may be presumed that the proteins are equally dispersed. The examination of the very large mitochondria of the *Amblystoma* liver shows that in that case the solids of the mitochondria though slightly irregular are largely disposed on the surface of the

unit, and that the central part of the unit is considerably less rich in organic substances. This fact may be demonstrated not only by the usual mitochondrial methods, but also in sections of frozen-dried material, denatured by alcohol or by heat in the absence of water, and stained with iron hematoxylin or acid fuchsin.

We thus arrive at a conception of the mitochondrion as a body whose organic substances are concentrated at its surface, this surface concentration consisting of a mosaic of protein and various lipoidal micellae. Such a structure has been suggested by Cowdry ('26), who thought that the surface of the mitochondria might be a monomolecular lipoidal film, and it is also supported by Bourne ('35). Bourne's diagram (*loc. cit.*, p. 241) would be acceptable except for the presumption that the surface layer of the mitochondria is composed exclusively of lipoidal and fatty molecules. The analyses of mitochondria contained in the preceding pages suggest that the main cortex of the mitochondrial unit is a mosaic of protein, glyceride and cholesterol molecules, rather than a lipoidal membrane.

This conception of the structure of mitochondria brings it into line with the coacervates of H. Bungenberg de Jong ('32), and it seems clear that mitochondria is a coacervate in the sense of this investigator.

The failure of mitochondria to blacken with osmic acid or to stain with Sudan III is a function of the dispersity of the fatty substances associated in this mosaic, and in addition a function of the water content of the granule in the natural state. It is clear that only in its lyophobic form does fat dissolve Sudan III or exhibit a visible blackening with osmic acid. When it is dispersed among other molecules or by a solvating medium, the degree of blackening or the degree of staining must of necessity be so diluted that it is not impressive under the microscope.

We have no means at present of knowing the water content of mitochondria. For certain reasons it is apparent that the water content is lower than that of the general mass of the cytoplasm. It may still be considerable. The reasons for

this point of view are: 1) the mitochondria are heavier than the other constituents of the protoplasm as demonstrated by ultracentrifugation by Beams and King ('34); 2) the intensity of the Millon reaction of mitochondria is greater than that of the intermediate substance of the protoplasm; 3) the principle of coacervation involves an intermolecular attraction which is greater than the repulsive force of the medium. Nevertheless, as shown by Lewis and Lewis ('15), the mitochondria are in osmotic equilibrium with the protoplasm and vary in volume in hypotonic and hypertonic solutions. This unknown water content is therefore an additional factor in so diluting the intensity of the Sudan III and osmic acid reactions that they become microchemically unimportant. Of course another reason is the extreme minuteness of the objects themselves. Dried mitochondria *en masse* blacken intensely with osmic acid and give an equally intense Millon reaction.

We may now consider the contradictions between the observations on the fresh cell and those on frozen-dried material discussed in an earlier paragraph. It is obvious that if, as just explained, mitochondria are coacervates in the sense of Bungenberg de Jong ('32), then the idea of a reversal of coacervation must be entertained. Any condition which disturbs the equilibrium of this coacervate might result in dispersion of its molecular units. The disappearance of mitochondria from cells during fixation by reason of the use of organic solvents or acid-fixing fluids is thus in all probability such a reversal.

If we concede that mitochondria is a coacervate, we must also consider the possibility that reversibility of coacervation may occur as the result of changes of equilibrium in the protoplasm itself. The disappearance and reappearance of mitochondria in living cells under observation, as described by Chambers ('24), however repugnant the idea may be to those who would elevate mitochondria to the dignity of living, self-reproducing units, must be definitely entertained as probable. There is nothing specific about the fats of mitochondria except their proportion to one another. Whether the proteins of the

mitochondria are specific, or whether they occur in the general ground substance in a dispersed form as well as in the coacervate form as mitochondria, cannot yet be determined. Further study of these proteins is thus clearly indicated.

It has been suggested by Joyet-Lavergne ('35) and by Bourne ('35) that the mitochondria contain glutathione and vitamine A. I am not able at the present time to express an opinion on these suggestions, since the preparations of mitochondria used in these analyses have already been washed several times in 0.85% sodium chloride solution and may therefore be presumed to have suffered some losses of substance.

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THE CULTIVATION OF ADULT RABBIT TESTICLE IN ROLLER TUBES ¹

WILLIAM MENDELSON

*Syphilis Division, Department of Medicine, Johns Hopkins University Medical
School, and Department of Embryology, Carnegie Institution of
Washington, Baltimore, Maryland*

ONE PLATE (THREE FIGURES)

The growth of tissue in vitro has been made possible by the use of a variety of methods, many of which possess advantages for a particular type of reasearch. Recently Gey ('33), Gey and Gey ('36) and Lewis ('34, '35) have described the roller tube method, in which an ordinary 150 × 16 mm. test tube is employed. This method offers a convenient technic for the study of the effects of drugs, toxins, viruses and hormones on the growth and viability of tissue cells. Relatively large amounts of tissue can be cultivated for long periods of time, with the production of unusually good growths of cells. The technic is comparatively simple and involves the use of nutrient media that are readily available in most laboratories.

The testicle of the adult rabbit provides an excellent source of living tissue cells in roller-tube cultures. Under sterile precautions, the testicle is removed and cut with small curved scissors into 2 mm. cubes in 0.85% salt solution. The test tubes are coated on the inside with 4 drops of heparinized chicken plasma, evenly disposed with a glass rod. The fragments of testicle are taken up in a small pipette and distributed along the inside of the tube. Usually two rows of

¹From the Syphilis Division of the Department of Medicine, Johns Hopkins University Medical School, and the Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland.

explants of six each are used, but the number of rows may be increased to four. Within 10 minutes the chicken plasma clots and forms a smooth lining which holds the explants firmly in place. One cubic centimeter of nutrient medium is then added and the tube is tightly closed with a sterile rubber stopper. The tubes are placed in a slightly inclined drum which rotates at 8 revolutions per hour inside an incubator at 37.5°C. The nutrient medium consists of 0.5 cc. of rabbit serum and 0.5 cc. of balanced salt solution (Gey, '33). Locke solution can be substituted for the balanced salt solution, and will give a satisfactory growth of cells, but better results are obtained with the balanced salt solution.

The growth and migration of cells begins early, progresses rapidly and reaches its maximum in 7 days. Within 24 hours a few isolated sprouts can be observed extending from the free edge of the explant. At this time the convoluted tubules, embedded in the thickness of the testicle explants, are barely visible. Within 48 hours, slender spindle-shaped cells begin to migrate out and form a radial growth around the entire explant. From this time on there is a progressive and rapid increase in growth and migration of cells, characterized by numerous mitotic divisions. For the most part, these cells are the fibroblasts and endothelial cells derived from the supporting tissue cells. As the stroma cells migrate away, the convoluted tubules become more prominent and their constituent epithelial cells begin to multiply. After 5 days the tubules are clearly outlined within the thickness of the explant. A tubule which earlier presented a free edge, is now continuous with a broad sheet of flattened and rhomboid polymorphous shaped cells. These are the germinal epithelium. A number of large cells with large nuclei were observed intermingled with the germinal epithelium; they appear to be similar to the cells described in fixed and stained sections as Sertoli cells. Occasionally, spermatozoa were seen in these cultures, they retained their motility for at least 48 hours and degeneration forms were observed as long as 12 days after growth in roller tubes. In the stained

preparations these are represented by tiny rods. The tails were not stained by hematoxylin. The spermatozoa may have been introduced as adult forms within the testicle explants; however, it is highly probable that maturation from spermatocytes takes place within the roller tubes.

After 7 days small areas of liquefaction form near the explant. It is necessary to patch these areas by relining the tubes with chicken plasma, which it clotted in the liquefied areas by means of a drop of chick embryo extract. Fresh supernatant fluid is then added. The cells continue to grow out in abundance (fig. 1). Cultures used in this investigation were maintained 21 days and patched, and the supernatant fluid was changed every 7 days. They can be maintained for longer periods if desired.

Permanent preparations of these cultures can be made by fixing and staining. The supernatant fluid is drained off as completely as possible and the tubes are washed with three changes of physiologic salt solution. Zenker's solution, containing 5% formalin, is used as the routine method of fixation, but any of the usual fixing solutions may be substituted. After 15 minutes the Zenker's solution is poured off, and the tubes are washed in three changes of water. The chicken plasma lining is then scraped away from the inside of the tube with a slender wooden applicator. It is desirable to leave an ample margin of chicken plasma clot surrounding the rows of explants. The preparations are subjected to the usual changes of alcohol, stained with hematoxylin, dehydrated and cleared in xylol. Because of the presence of the plasma clot, differential staining seldom proves to be satisfactory. About 3 cc. of balsam is placed in the tube and a Wasserman tube, filled with cedar oil and closed with a cork stopper, is then allowed to slide into the culture tube. The balsam rises and fills the space between the two tubes just as it does between a slide and a cover glass. The excess of balsam continues to rise about the level of the Wasserman tube and, when dry, forms a permanent seal.

The large growth of cells obtained in roller tube cultures of the testicle from the adult rabbit has been used to test the toxicity of a few arsenical drugs (unpublished results). The actively growing explants of the testicle have also served as a culture medium in an attempt to grow the *Spirochaeta pallida* (Eagle and Mendelsohn, in press). This method is especially suited for the study of the viability of living cells during exposure to certain hormones, toxins and viruses. It may be possible to recover from the supernatant fluid of these tissue cultures certain products of metabolism, such as hormones.

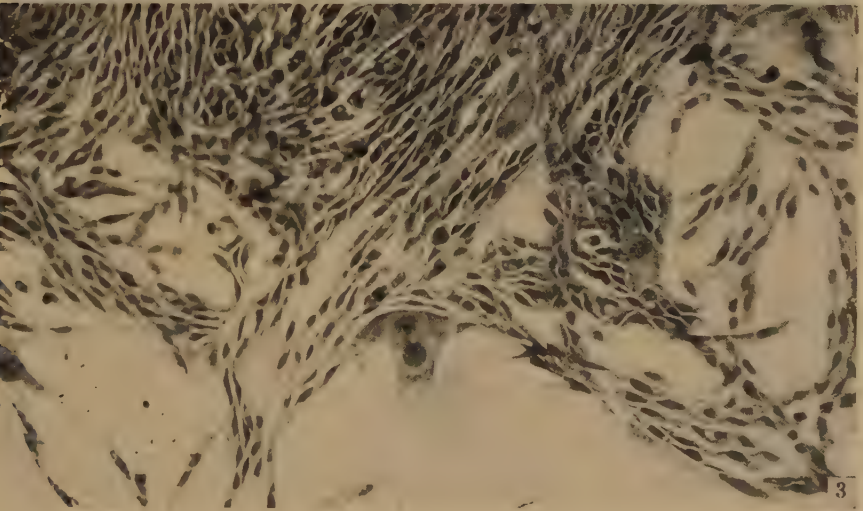
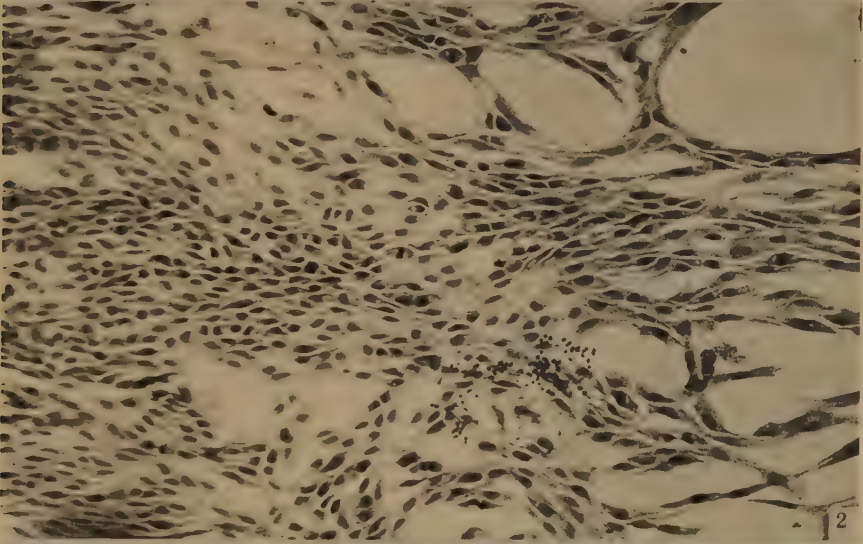
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PLATE 1

EXPLANATION OF FIGURES

- 1 Living explant of testicle showing extensive migration and growth of cells. Twenty-one-day culture. $\times 60$.
- 2 A sheet of germinal epithelium with a group of spermatozoa shown as small dark rods. Twelve-day culture fixed and stained. $\times 150$.
- 3 Germinal epithelium and large Sertoli cells. Twelve-day culture fixed and stained. $\times 150$.



THE VASCULAR SUPPLY OF THE HYPOPHYSIS CEREBRI OF THE CAT

GEORGE B. WISLOCKI

Department of Anatomy, Harvard Medical School

SIXTEEN FIGURES

An account of the blood supply of the pituitary gland of the rhesus monkey has appeared recently (Wislocki and King, '36; Wislocki, '37 c). In view of the unusual relationships of the blood vessels of the hypophysis reported for the monkey, it was considered of importance to turn to some other mammal for purposes of comparison. Consequently the blood vessels of the hypophysis of the cat have been investigated, an account of which is given in the present communication.

Previous investigations of the blood supply of the cat's pituitary are confined to the observations of Herring ('08) and Stevens ('37). However, the hypophyseal vessels have been described in another carnivore—the dog—by both Dandy and Goetsch ('11) and Basir ('32). Comparison of the observations of these previous workers with our own will be presented in a discussion which follows the description of the present material.

MATERIAL AND METHODS

We have investigated the pituitary of the cat by two methods. The first consisted in the dissection, under a low power dissecting microscope, of the pituitary region in which the blood vessels had been previously injected with India ink. For this purpose the injected heads with the skull opened were fixed in 10% formalin. Subsequently the hypophyseal region was exposed by dissecting away the base of the skull with rongeurs. The brain was then transferred from formalin to 70% alcohol in which the further dissection of the hypophy-

sis was carried out under a dissecting microscope. Figures 1 to 4 illustrate the results of this technique.

The second method consisted of the preparation of serial sections through the hypophysis left in situ in the sella turcica and with the blood vessels filled by injection with India ink. Figures 5 to 15 illustrate the character of the sectioned material.

The details of injecting the hypophysis and preparing the sections are as follows: Higgins' waterproof India ink, diluted one-half with distilled water, was injected through the heart into several freshly killed cats. The head, with the top of the skull removed, was fixed in 10% formalin. Subsequently the base of the brain with the hypophysis in situ in the sella turcica was decalcified in 4% trichloroacetic acid. The specimens were then embedded in celloidin and cut serially in $200\ \mu$ sections or alternately in $200\ \mu$ and $20\ \mu$ sections. The $200\ \mu$ sections were cleared and mounted unstained in balsam. The $20\ \mu$ sections were stained and similarly mounted. Serial sets were prepared in both sagittal and frontal planes.

Combining information gained from dissection of the pituitary in the manner described with that derived from study of serial sections of pituitaries offers considerable opportunity for elucidating the extraordinarily complex arrangement of the hypophyseal vessels.

OBSERVATIONS

The results of the study of the cat's hypophysis are best presented by constant reference to the accompanying drawings and photographs. The photographs do not render all of the detail and beauty of perspective of the original sections viewed under a low power binocular microscope, but, nevertheless, they achieve the purpose of bringing out a number of salient features.

The vessels will be described under three main headings: the arterial supply, the portal veins, and the systemic veins.

The arterial supply. The hypophysis of the cat is supplied by two main sets of arteries which for convenience are termed the superior and the inferior hypophyseal arteries.

The superior hypophyseal arteries consist of a variable number of small arteries given off from the internal carotid arteries near their junction with the circle of Willis, or directly from the vessels of the circle of Willis. These arterioles are well seen in the dissection presented in figure 1. They pass, either directly or by first dividing, into the pituitary,

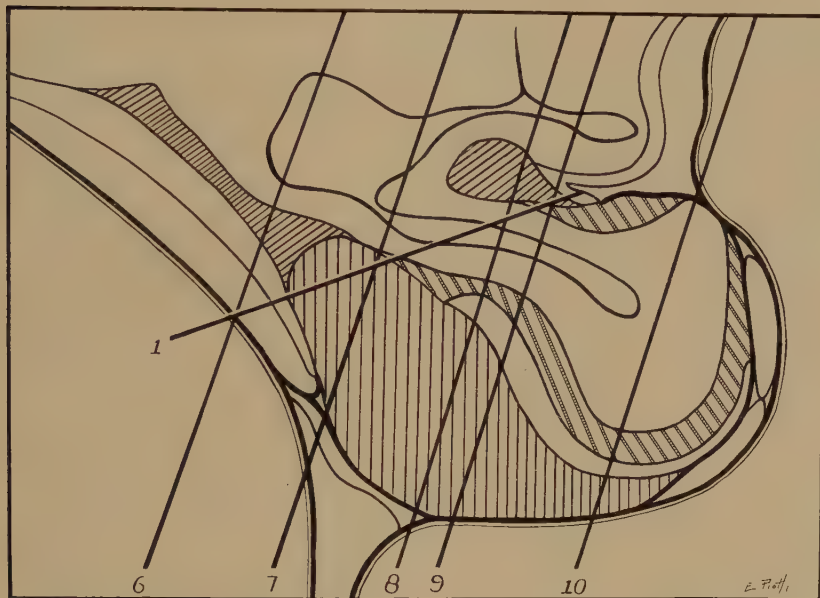


Fig. A Schematic outline of the hypophysis of the cat. Line 1 indicates the plane at which the hypophysis was cut in the dissection shown in figure 1. Lines 6 to 10 indicate the planes at which the successive frontal sections shown in photographs 6 to 10 are located. Neural lobe, plain white. Anterior lobe, perpendicular hatching. Pars tuberalis, narrow oblique hatching. Pars intermedia, wide oblique hatching.

penetrating the mantle of pars tuberalis surrounding the stalk. Some terminate locally in the stalk, while others pass along the stalk to enter the anterior lobe. The plane of the hypophysis shown cut by the knife in figure 1 may be better understood by reference to figure A (plane 1) which illustrates its relation to the stalk and anterior lobe. From examination of figure 1 it is clear that some ten to fifteen arterial

twigs reach the stalk and anterior lobe in the manner indicated.

An interesting vessel, differing in some respects from the others, is one that is shown lying in the pia arachnoid and skirting the stalk posteriorly (fig. 1, a1). An arteriole from each side anastomoses here in the midline, creating a communicating arteriole which gives off one or two branches to the posterior surface of the stalk. Besides these, it gives off a branch (a2) which has been found in two dissections, as well as in sectioned material, and which penetrates the processus infundibuli from above. This seemingly constant vessel is shown in figures 1 and 5 (a2).

Thus the superior hypophyseal arteries in the cat supply the stalk and anterior lobe as a series of small arterioles, but also, by virtue of an anastomosis posteriorly, provide a constant, median branch which penetrates the processus infundibuli.

The inferior hypophyseal arteries are given off from the internal carotid arteries as the latter traverse the cavernous plexuses. The inferior hypophyseal arteries and their relations to the hypophysis are clearly seen in the dissection shown in figure 2. A branch from the carotid on either side passes medially to anastomose in the midline with its fellow from the opposite side. From this communicating trunk several

ABBREVIATIONS FOR ALL FIGURES

ant, anterior lobe	me, median eminence (eminentia saccularis) of the tuber cinereum (Tilney)
a1, communicating arteriole belonging to the group of superior hypophyseal arteries	mr, ms, mammillary recess
a2, branch of communicating arteriole (a1) supplying the processus infundibuli	pt, pars tuberalis
cs, ramus of cavernous sinus	pv, portal veins
hc, hypophyseal cleft	sas, subarachnoid space
iha, inferior hypophyseal arteries	sd, subdural space
inf, infundibulum	sha, superior hypophyseal arteries
int, pars intermedia	vpd, systemic veins draining the anterior lobe
ir, infundibular recess	vpi, systemic veins draining the processus infundibuli and the pars intermedia
	V III, third ventricle

branches are given off which penetrate the posterior pole of the neural hypophysis, sinking at once into the interior of the infundibular process. These arteries constitute the main source of blood of the neural lobe. They can be seen clearly in figures 2, 5 and 10 (iha). The inferior hypophyseal arteries anastomosing in the midline and giving off branches to the



Fig.1 Hypophyseal region of cat dissected, showing the base of the brain and the pituitary stalk, with the body of the hypophysis cut off in the plane marked 1 in figure A. The dissection shows the manner in which the hypophysis is supplied with blood by the superior hypophyseal arteries which arise from the internal carotids and the circle of Willis. Posterior to the hypophyseal stalk, an arteriole from each side, belonging to the group of superior hypophyseal arteries, anastomoses with its partner to form a communicating arteriole (a1) which gives off twigs to the stalk, as well as a vessel (a2) which enters the infundibular process.

neural hypophysis should not be confused with the similar, but smaller, communicating arteriole derived from the superior hypophyseal arteries described above, which gives off a single median branch to the upper aspect of the neural pole.

The above account outlines briefly the arterial blood supply of the pituitary of the cat. It should be added that examination of a number of dissected as well as sectioned hypophyses indicates a rather wide latitude in individual pattern of the

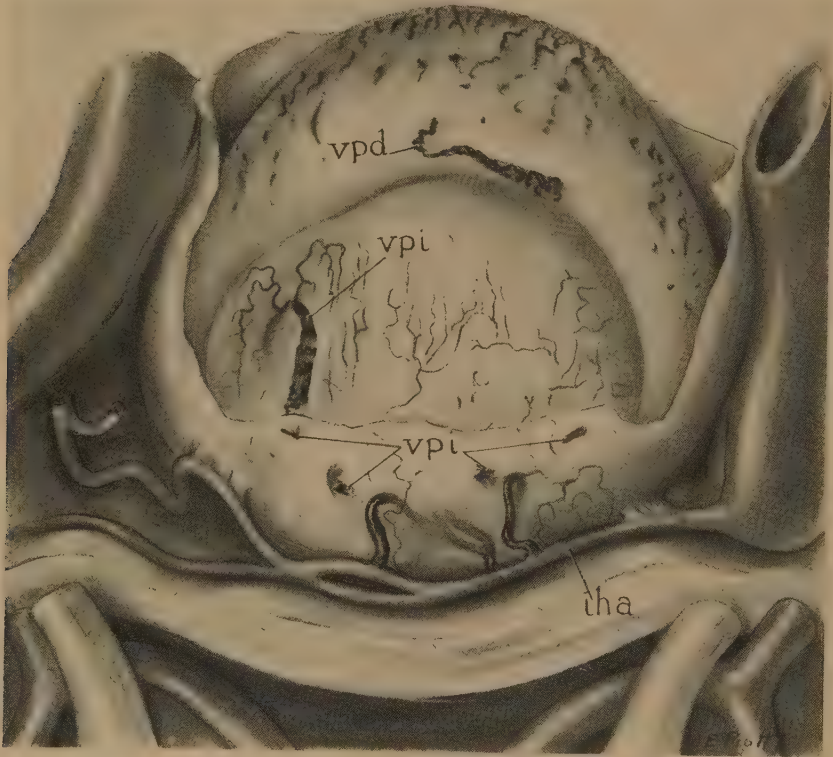


Fig. 2 Dissection showing the pituitary viewed from behind. The inferior hypophyseal arteries (iha) are seen approaching the neural pole from either side and anastomosing in the midline. Near the midline several branches are given off which immediately penetrate the interior of the neural process. The posterior hypophyseal veins (vpi) are seen emerging from the infundibular process and piercing the wall of the intercavernous sinuses. Anteriorly one of the efferent veins of the anterior lobe is seen (vpd) leaving the gland to become a flattened dural vein—an offshoot of the cavernous plexuses.

arteries arising from the carotids and circle of Willis to supply the hypophysis. For example, the slender artery, seen in figure 1, which passes forward on the left side from the carotid into the notch between the optic nerves is, in one of the dissections, a very much larger vessel which anastomoses in the midline, just anterior to the hypophyseal stalk, with a vessel of similar size taking origin from the carotid on the opposite side. Furthermore, it has been observed that the arterioles which meet to create an anastomosis posterior to the stalk (fig. 1, a1) may be of unequal size, the contributing branch on one side preponderating markedly over its partner. Similarly the inferior hypophyseal arteries (fig. A, iha) may vary somewhat—the vessel on one side may be larger than its partner. Moreover, one or both contributing vessels may be single, or in places double, and may arise variably as one or two offshoots from the carotid artery. These anatomical variations, however, do not change essentially the fundamental distribution which has been outlined above.

We have traced the arteries from their origins to the various parts of the pituitary. It is necessary to attempt to trace their distribution within the hypophysis. The arterioles which reach the pituitary stalk break up in part locally into a rich plexus of wide calibered capillaries which can be seen in figures 4, 5, 6, 7, 11 and 15. This plexus lies in the substance of the pars tuberalis which forms a mantle around the infundibular stalk. From this mantle minute plexiform tufts of capillaries project to a variable degree and with inconstant distribution, as examination of different specimens reveals, into the interior of the infundibular stalk (figs. 6, 7, 11, 12 and 14). On the whole the vascularization of the interior of the infundibular stalk is relatively scanty, although the surface plexus in the enveloping pars tuberalis is extremely rich.

Besides vascularizing the hypophyseal stalk in the manner outlined, a number of branches of the superior hypophyseal arterioles pass along the stalk on the surface of the pars tuberalis to enter the substance of the pars distalis where they are distributed to the characteristic sinusoids of this portion of the gland.

The branches of the posterior hypophyseal arteries, as well as that peculiar branch of the superior hypophyseal arteries supplying the upper aspect of the neural lobe (figs. 1 and 5, a1), on entering the processus infundibuli, penetrate at once into the interior of the organ (figs. 2, 5 and 10). There they break up into a rich bed of relatively wide-calibered capillaries supplying the substance of the infundibular process. The pars intermedia (figs. 5, 11 and 12, int) invests the bulk of the neural process, the two being separated almost completely from the anterior lobe by an extensive, persistent hypophyseal lumen. In consequence of the close adherence of the pars intermedia to the neural tissue, it derives its blood supply exclusively from the latter. It is relatively sparsely supplied with blood vessels, possessing only a network of capillaries which derive their blood from the small arterioles in the interior of the processus infundibuli and which drain, on the other hand, into venules which collect characteristically at the boundary between the neural process and the pars intermedia. The features described here can be seen in figures 5, 11 and 12.

Portal veins. We believe that we have discovered hypophyseal portal venules in the cat similar to the ones occurring in the rhesus monkey (Wislocki and King, '36). They connect the rich capillary plexus of the pars tuberalis which surrounds the infundibular stalk, with the anterior lobe. Portal vessels connecting the capillary bed of the pars tuberalis with the sinusoids of the pars distalis (anterior lobe) can be traced in the type of serial sections shown in figures 7, 8, 14 and 15. Nevertheless, because of the complex curvature of these vessels, imposed by the shape of the hypophyseal stalk, the constricted neck, and the expanded body of the anterior lobe, it is difficult to demonstrate the entire course of an individual portal venule in a satisfactory manner. However, in the course of dissecting the pituitary by the technique cited in an earlier paragraph, we discovered a way of showing the portal venules quite clearly. Figure 4 shows the preparation under discussion. The hypophyseal stalk has been separated from the diencephalon. This is accomplished easily by gentle

traction, the line of separation occurring naturally between the pars tuberalis and the median eminence (*eminentia saccularis*) of the tuber cinereum,¹ on the one hand, and the hypothalamus on the other. After separation the hypothalamus is exposed as shown in the center of figure 3. The detached hypophyseal stalk is next oriented under the dissecting microscope with its interior facing the observer. It is necessary next to remove the infundibular stalk composed of neural tissue which can be easily pulled away from the mantle of pars tuberalis by the aid of fine forceps. This procedure leaves the mantle of the pars tuberalis intact in the form of the preparation shown in figure 4, in which the rich, vascular plexus of the pars tuberalis can be seen to advantage. Here the portal venules can be seen with great clarity. They take origin from the periphery of the thin shell of pars tuberalis increasing in caliber toward the center of the preparation where they converge disappearing from view in the region of the constricted neck into the tissue of the pars distalis. Segments of these venules, less clearly defined, can be seen in figures 5, 7, 8 and 14.

In consequence of study of our preparations we are convinced of the existence of portal venules in the cat, extending from the hypophyseal stalk to the anterior lobe. Moreover, we find no evidence in our preparations that these venules arise from or penetrate into the hypothalamus proper; they are confined solely to the hypophyseal stalk. For example, in figure 3 no vessels, either venous or arterial, of any size or constancy, are visible in the wall of the hypothalamus from which the hypophysis has been detached.

Furthermore, we find no evidence that these portal venules have any direct connections as veins with the infundibular process and its mantle of pars intermedia. The infundibular process possesses fairly abundant capillary connections with

¹ We are discarding the term *eminentia saccularis* of the tuber cinereum and, for reasons presented by Tilney (*Bull. Neur. Inst. of New York*, 1936, vol. 5, pp. 387-436), are adopting instead the designation median eminence for the median infundibular portion of the tuber cinereum.



Fig. 3 Preparation of the hypophyseal region, derived, by further dissection, from the specimen illustrated in figure 1. The hypophyseal stalk has been removed revealing its site of attachment to the hypothalamus. The arteries of the circle of Willis and their branches have been dissected away uncovering the basilar veins and venules on the brain surface. The dissection reveals that no arteries or veins of any consequence pass from the hypophyseal stalk into the hypothalamus. It shows also that a few minute venules arise circumferentially from the border (pars tuberalis) of the hypophyseal stalk.



Fig. 4 Dissection of the hypophyseal stalk obtained by detaching the stalk from the brain, the plane of separation being shown in figure 3. The detached stalk, turned so that one views the interior, has had the neural stalk plucked away under the dissecting microscope with fine forceps, leaving intact the mantle of the pars tuberalis with its rich plexus of capillary sinusoids. Around the periphery of the specimen a few tags of arterioles (sha) can be seen. In the interior of the pars tuberalis one sees plainly a number of portal venules arising from the periphery of the pars tuberalis and following a course toward the central part of the specimen where the veins disappear from view by penetrating the anterior lobe.

the hypophyseal stalk, but none by actual arteries or veins. Moreover, excepting capillary anastomoses in the neighborhood of the neck of the pituitary, the blood supply of the infundibular process in the cat is practically independent of that of the anterior lobe. These observations are readily substantiated by reference to figures 6 to 10 which are successive frontal sections through the hypophysis, the orientation of each of which is understandable by reference to figure A. The key section to the question under discussion is shown in figure 9. Here one can examine the total extent of the anatomical connections between the infundibular process, on the one hand, and the stalk and anterior lobe on the other. One can observe the infundibular recess (ir) surrounded by the distal portion of the infundibulum (inf), in turn surrounded by the pars intermedia (int), in turn surrounded by the hypophyseal cleft (hc). The stalk is connected above with the anterior lobe by a thin lamina on either side. Should essential arterial or venous connections between the infundibular process and the other parts of the pituitary exist, they should of necessity have to be present in this section. One finds, however, no evidence of such connections. It can be observed that the pars intermedia is vascularized by relatively sparse capillaries which radiate from an annular network of minute vessels of practically capillary dimensions located at the interface between stalk and intermedia. This plexus, if followed anteriorly (fig. 8), becomes confluent with the plexus of the pars tuberalis; if followed posteriorly (fig. 10), it connects with the rich plexus of the infundibular process. Thus vascular continuity exists between stalk and infundibular process,

Fig. 5 Somewhat schematized drawing of a thick median sagittal section through the cat's hypophysis. This shows clearly the superior hypophyseal (sha) and the inferior hypophyseal (iha) arteries, the portal venules (pv), the systemic veins draining the anterior lobe (vpd), as well as the veins draining the neural lobe (vpi). It shows also the communicating arteriole (a1) behind the stalk and the branch (a2) which it sends into the upper part of the neural lobe. The section illustrates also the relative vascularity of the different parts of the gland, including the pars intermedia. It illustrates also the absence of communication with the hypothalamus, excepting by capillaries.



but it is purely of a capillary anastomotic order and does not involve the passage of actual portal venules from the one locality to the other.

In view of these findings it appears reasonable to conclude that the portal veins are restricted to connections between the stalk and the anterior lobe. The further question of whether the direction of the flow of blood in the portal veins is from stalk to anterior lobe or vice versa we believe can be answered by anatomical evidence which will be presented in the following section describing the systemic veins draining the pituitary.

Systemic veins. As the result of our observations, we believe there are two major routes for escape of the blood by systemic veins from the pituitary gland of the cat. One set of these veins drains the anterior lobe, the other the neural lobe; both sets of draining veins pass from the body of the gland into the adjacent cavernous sinuses. Figures 2, 5 and 11 illustrate the veins (vpd) which drain the anterior lobe.

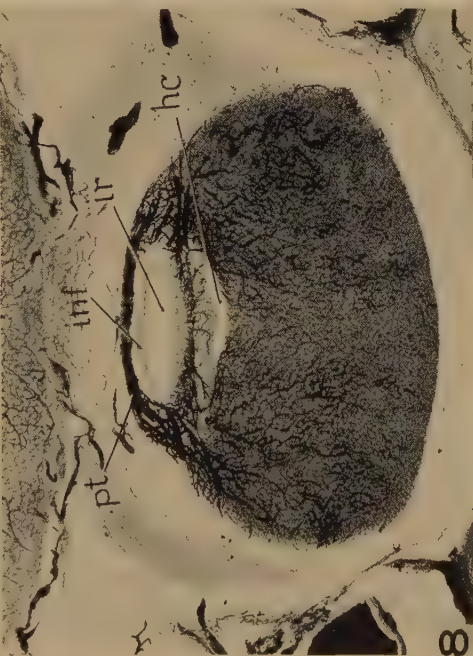
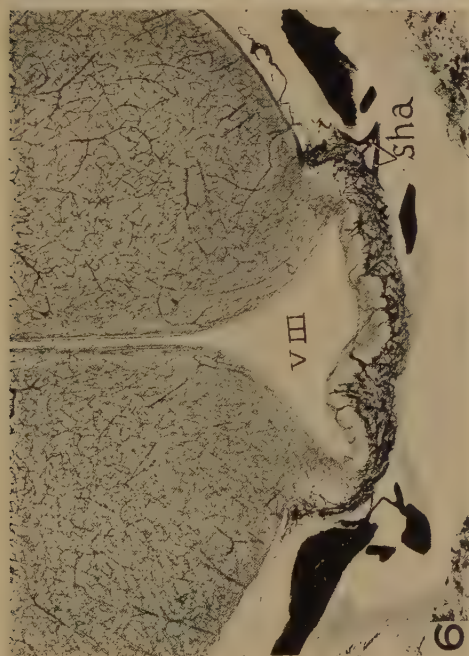
Figures 6 to 10 are photographs of successive frontal sections through the hypophysis of the cat. The orientation of the planes of these sections is further explained by reference to figure A in which the lines numbered 6 to 10 show the location of the sections.

Fig. 6 Shows the anterior part of the hypophyseal stalk with the richly vascular pars tuberalis covering the thin wall of the infundibulum. Twigs of the superior hypophyseal arteries can be seen entering the pars tuberalis. The infundibulum is relatively avascular, excepting small tuft-like extensions into it of the plexus of the pars tuberalis. $\times 20$.

Fig. 7 Shows the infundibulum at the level of the appearance of the anterior lobe. Here segments of portal venules are visible, connecting the pars tuberalis and the anterior lobe. In neither this nor the preceding section is there evidence of the passage of veins from the stalk into the hypothalamus. $\times 20$.

Fig. 8 Presents a section somewhat farther back showing the constricted infundibulum surrounded by pars tuberalis. Vessels are passing from the pars tuberalis into the anterior lobe. $\times 20$.

Fig. 9 Represents a section in which the infundibular recess has narrowed down to a circular lumen surrounded by the infundibulum and this in turn by pars intermedia. A pronounced condensation of capillaries exists between the infundibulum and the mantle of intermedia. From this plexus radial capillaries penetrate the intermedia. This section illustrates the absence of any sizable arterioles or venules connecting the anterior lobe and stalk, on the one hand, with the neural process; if larger vessels were to do so, they should be visible in this section of the intervening zone. $\times 20$.



These venules arise within the anterior lobe and unite to form larger trunks which pass backward and downward to reach the surface of the gland. They pass from the surface of the gland into the adjacent, encapsulating dura where they connect with the ramifying system of the cavernous sinuses. The systemic veins of the neural lobe arise as a system of venules lying between the processus infundibuli and the pars intermedia and collecting blood from these two tissues. The veins of the neural lobe pass backward on the surface of the processus infundibuli to emerge from the pituitary at its posterior pole as venules which enter the cavernous sinuses. The course and relationships of these veins draining the processus infundibuli and pars intermedia are well shown in figures 2, 5, 12 and 13 (vpi).

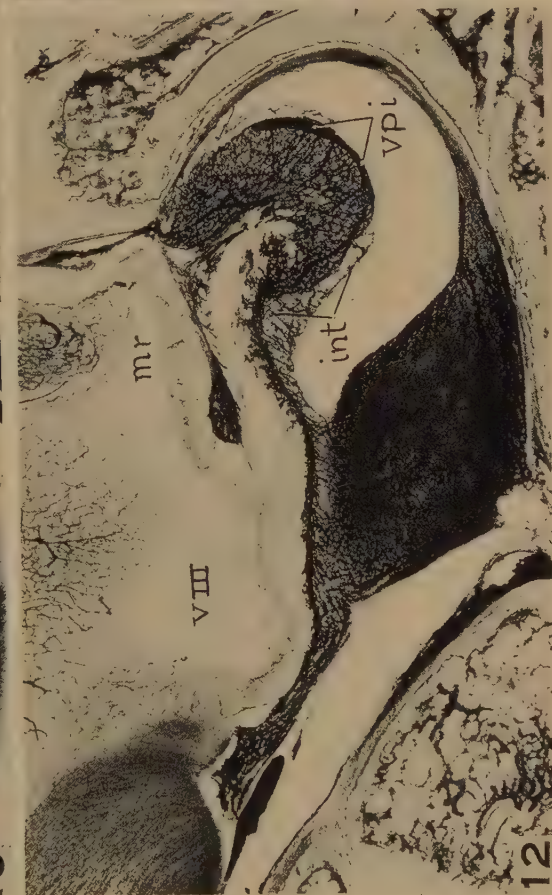
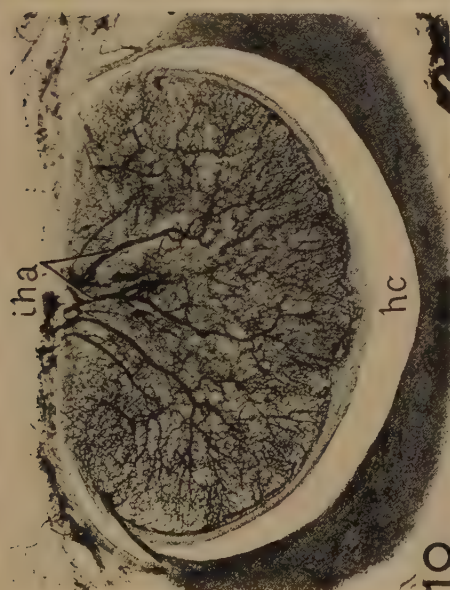
The question arises as to whether these two sets of veins constitute the essential drainage of the pituitary, or whether there are other veins draining upward from any part of the hypophysis into the brain or into the system of the basilar veins. We must answer the possibility of connections with the brain in the negative, because we have not found any veins from the neural stalk traversing the median eminence of the tuber cinereum to reach the hypothalamus. Figures 3, 4, 5, 6, 7, 12, 14 and 15 support this conclusion. The connections between hypothalamus and hypophyseal stalk are practically all of the caliber of capillary anastomoses. In figures 14 and

Fig. 10 Shows a section through the infundibular process illustrating how branches of the inferior hypophyseal arteries penetrate the neural lobe. The thin shell of pars intermedia enclosing the neural process is almost devoid of vessels excepting a few capillaries. $\times 20$.

Fig. 11 Median sagittal section through the cat's pituitary presented to show the systemic veins (vpd) leaving the anterior lobe. $\times 20$.

Fig. 12 Median sagittal section given to illustrate the relative vascularity of the different portions of the gland. A portion of one of the systemic veins of the neural lobe, peripherally situated between the neural lobe and the pars intermedia, is also visible (vpi). $\times 18$.

Fig. 13 Sagittal section showing systemic veins (vpi) of the neural process joining and about to enter the nearby intercavernous sinus (cs). $\times 20$.

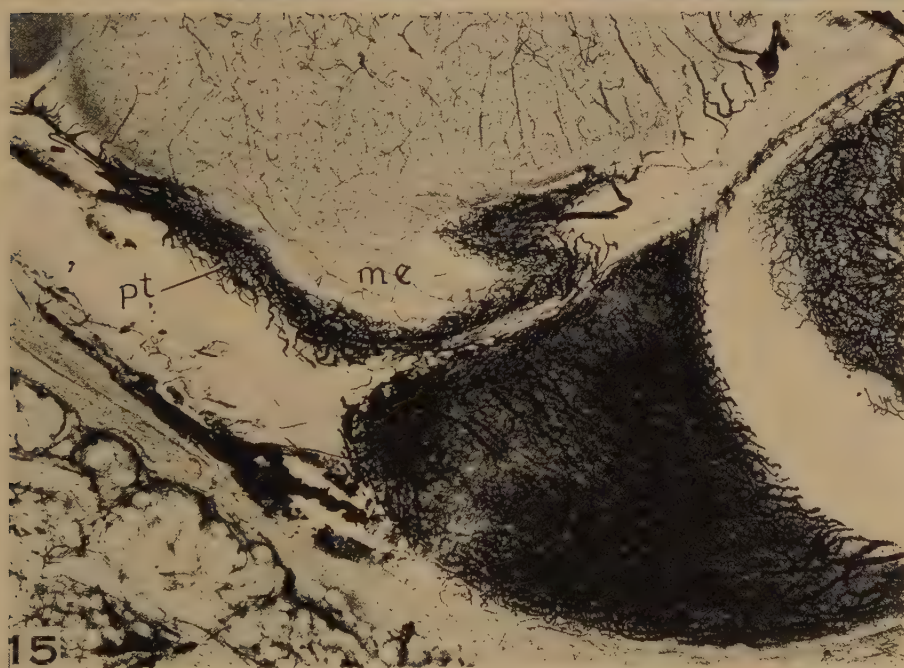


15 several vessels which are larger than capillaries emanate from the outskirts of the pars tuberalis and penetrate the hypothalamus for a short distance; dissection of the region reveals, however, that these occasional vessels are small twigs of the superior hypophyseal arteries.

Regarding the possibility of systemic drainage from the pituitary into tributaries of the system of basilar veins, the dissection shown in figure 3 supplies the answer. If one dissects away the hypophyseal stalk and the arteries at the base of the brain, one arrives at a dissection as seen in figure 3. The stalk, as exemplified in figure 4, has been lifted off, exposing the attachment of the stalk to the floor of the third ventricle (fig. 3). After dissecting away the arteries, the underlying system of pial venules on the surface of the base of the brain is now exposed to view. From study of figures 3 and 4 one cannot gainsay that a certain amount of capillary drainage occurs from the extreme edge of the pars tuberalis into the basilar system, but we do not regard this drainage as of great significance compared with the sizable systemic channels demonstrated leaving the body of the pituitary to communicate with the cavernous sinuses.

Fig. 14 A sagittal section to show the dense vascularization of all portions of the hypophysis except the pars intermedia (int), the wall of the infundibulum (inf) and median eminence of the tuber cinereum (me). $\times 28$.

Fig. 15 A parasagittal section through the region of the pituitary stalk, showing the tremendous vascularity of the pars tuberalis and the relative avascularity of the median eminence of the tuber cinereum (me). This and preceding sections (figs. 6, 7, 12 and 14) also illustrate the absence of veins passing from the region of the anterior lobe through the stalk into the hypothalamus. All of the vessels connecting the stalk region with the hypothalamus are capillaries, excepting two larger vessels occurring in figure 14 and two more in figure 12 which are arterioles, branches of the superior hypophyseal arteries which pass through part of the pars tuberalis to enter the hypothalamus. $\times 28$.



DISCUSSION

From study of the material which we have at our disposal, we have reached several conclusions regarding the blood supply of the hypophysis of the cat.

In the first place, we have found the circulation in the pituitary gland to be practically independent, except for capillary anastomoses, from that of the brain proper. The blood vessels supplying the stalk and anterior pituitary are of pial origin, but they were preempted during embryonic growth by the epithelial hypophysis, a process which deflected them from their original destination and rendered them independent of the vessels providing the neighboring hypothalamic area with blood. Furthermore, the processus infundibuli, in sharp distinction from the rest of the brain, has derived its blood supply quite independently from dural arteries in the wall of the sella turcica. Detailed observations and a discussion regarding these points of embryology of the pituitary blood vessels have been presented in a previous paper (Wislocki, '37 a and b).

Secondly, our results indicate that in reference to supply by arteries and drainage by veins the stalk and anterior lobe on the one hand, and the infundibular process and pars intermedia on the other, are also almost independent of one another. By virtue, however, of the continuity of the infundibular stalk with the infundibular process, the capillary plexus surrounding the former is in anastomotic continuity with the vascular bed of the latter, but this involves solely intercommunication by capillaries.

In the third place, we have adduced evidence for the existence of portal venules connecting the rich plexus of the pars tuberalis, surrounding the infundibular stalk, with the sinusoids of the anterior lobe. In consequence of our findings we regard the sinusoids of the anterior lobe as being fed with blood by both arterioles and portal venules, much in the manner of the hepatic sinusoids. Furthermore, the blood from these hypophyseal sinusoids drains through systemic venules into the nearby cavernous sinuses.

Further substantiating evidence for our second and third theses can be derived from consideration of material in which the blood vessels of the pituitary gland have not been completely filled with India ink. It is fairly difficult and often requires a number of trials to achieve a complete filling of the vascular bed of the pituitary. Fortunately pituitaries in which the blood vessels are incompletely injected contribute valuable evidence on two points. Examination reveals in many of these specimens that the neural lobe is fully injected, whereas the vascular bed of the anterior lobe is not injected at all or only slightly so. Very rarely the opposite is encountered, namely, fairly good filling of the anterior lobe and little or none in the infundibular lobe. These results indicate that the blood supplies of the neural and anterior lobes are in the main independent of one another.

The other point revealed by partial filling of the vessels pertains to the stalk and pars distalis. The most unsuccessful injections fill without exception the vessels of the hypophyseal stalk. The anterior lobe, on the contrary, is always the most difficult portion of the gland to fill; however, when it does become injected, the injection mass appears more readily in the region contiguous to the stalk than at the lower or venous pole of the anterior lobe—a region exceedingly difficult to penetrate. This behavior is a further indication of the correctness of the concept outlined above, namely, that the anterior lobe possesses an arterial pole related to the stalk and a venous pole from which the efferent veins emerge to enter the dura.

We come finally to a brief survey of the previous literature regarding the blood supply of the pituitary of the cat and the dog. Herring ('08) gives a short account of the vascular supply of the cat's pituitary body. His description differs from our own in many particulars, involving on his part no mention of portal venules, nor a description of the veins we have found draining the anterior lobe. Regarding the neural lobe our observations agree in the main with his. He describes arteries penetrating and veins emerging from the neural pole

of the gland, giving the posterior lobe in his estimation a blood supply independent of that of the anterior lobe. The blood to the latter he regards as coming down through the stalk. Furthermore, of the anterior lobe he says that it is one of the most vascular structures in the body, whereas the posterior lobe resembles, in the number and arrangement of its vessels, the adjacent white matter of the brain.

Stevens ('37) has recently investigated the blood supply of the cat's pituitary by quantitative measurements of the total lengths and average diameters of the capillaries in unit blocks of tissue. She found that the anterior lobe of the hypophysis of the male cat is more richly vascularized than the posterior lobe, the ratio being 6:1. The average diameter of the vessels in the anterior lobe is twice that of the posterior lobe. The pars intermedia is relatively poor in capillaries; the pars tuberalis, on the other hand, is richly supplied with blood sinusoids. In this study the actual origins and connections of neither arteries nor veins were investigated.

In another carnivore, the dog, Dandy and Goetsch ('11) have traced the blood supply of the pituitary. The superior hypophyseal arteries converge symmetrically from all quadrants of the circle of Willis toward the hypophyseal stalk. In our preparations of the cat, on the contrary, these vessels are fewer in number and are much more devious in course and irregular in the manner in which they anastomose. Moreover, in figure 2 in the paper by Dandy and Goetsch it is a misnomer to apply the term *A. communicans ant.* to the vessels so labelled. It is agreed anatomically that the artery so designated lies anterior to the optic chiasma. Further, they make no mention in the dog of a system of portal venules. The veins draining the anterior lobe, according to them, pass along the stalk to a venous circle immediately overlying the circle of Willis and draining into the *venae magnae Galeni*. In the cat such connections are quite subsidiary, the principal drainage being into venules entering dural rami of the cavernous sinuses. At the time their paper was written they did not discriminate between pars tuberalis and pars intermedia.

They say simply that the pars intermedia derives its supply from the vessels of the stalk, from the adjacent brain, and from the posterior lobe. Newer knowledge regarding the relations of pars tuberalis, infundibulum, and pars intermedia, as well as the discovery of the hypophyseal portal system, makes a clearer analysis possible at the present time of the vascular relationships in the interior of the gland.

The most recent description of the blood supply of the hypophysis of the dog is presented by Basir ('32). He has adopted the concept of hypophyseal portal veins as enunciated by Popa and Fielding ('30) for man. These writers believe that they have demonstrated hypophyseal portal venules passing from both anterior and posterior lobes of the pituitary via the pituitary stalk to the nuclei of the tuber cinereum and the other parts of the hypothalamus. Consequently they ascribe to this system of veins the role of conveying blood from both parts of the body of the pituitary to hypothalamic brain centers. The present investigator regards the course and connections of these venules, as well as the role which they play in the hypophyseal circulation, as totally different from the description and function assigned to them by either Popa and Fielding or Basir. Our disagreement is based upon the findings derived from the present study of cats, as well as from previous investigations on monkeys (Wislocki and King, '36; Wislocki, '37 c). From our own investigations we regard the hypophyseal portal venules as vessels arising locally in the hypophyseal stalk and conveying blood to the anterior lobe. Adequately injected material of both monkey and cat indicates that the pituitary is practically independent of the hypothalamic nuclei as far as linkage by veins is concerned. Hypothalamus and pituitary stalk are connected by capillary anastomoses only. Furthermore, preparations, especially in the cat, demonstrate practically complete independence of the circulation of the processus infundibuli (posterior lobe) from that of the anterior lobe and connections by capillaries only with that of the hypophyseal stalk. In view of the above findings, coupled with the discovery of important venules

which drain from the anterior lobe into the cavernous sinuses, the conclusion has been reached that the portal venules convey blood from the pars tuberalis and the infundibular stalk to the anterior lobe of the pituitary.

In conclusion it is of interest to compare the results obtained in the present study on the cat with the previous findings in the monkey. The two forms agree in many major particulars: for example, the presence of portal venules connecting the stalk with the anterior lobe, the demonstration of systemic veins from the anterior lobe communicating with the cavernous sinuses, the absence of vascular connections, excepting capillaries, between the hypophysis and the brain centers of the hypothalamus, the marked degree of independence of the pituitary circulation from the blood supply to the brain proper, and the relative independence of the circulations of the anterior lobe and neural process.

The main differences between the cat and the monkey should also be emphasized. Perhaps the chief difference pertains to the degree of vascularization of the infundibulum and the median eminence (*eminentia saccularis*) of the tuber cinereum.² In both animals it has been pointed out that curious arborizing tufts of blood vessels, mainly large capillaries, penetrate the neural stalk from the surrounding mantle of pars tuberalis. In the monkey these tufted plexuses penetrating the stalk are much more abundant and larger, especially in the region of the median eminence, which by virtue of its abundant supply of such vessels is sharply delimited from the brain proper. In the cat the median eminence is relatively avascular, and consequently shows no such sharp boundary on the score of vascularity between it and the rest of the brain. Since we have accepted in the previous study on the monkey that these arborizing plexuses of the median eminence drain into the portal system of the pars tuberalis, it follows that in the cat the median eminence is somewhat subsidiary in the amount of blood delivered to the portal system. In the latter animal the portal system arises mainly

² See footnote 1 on page 369.

within the confines of the pars tuberalis, the median eminence representing a relatively avascular zone separating the extremely vascular pars tuberalis of the hypophysis from the brain centers of the hypothalamus above.

Other differences between the cat and the monkey are noteworthy. It should be mentioned that the systemic veins draining from the anterior lobe into the cavernous sinuses are situated laterally in the monkey, medially in the cat. Furthermore, we have found an arteriole, constant in occurrence in the cat, which enters the upper surface of the processus infundibuli. This we have not observed in the monkey, but it must be borne in mind that we discovered this arteriole principally by the technique of dissection which we have employed more extensively in the cat than in the monkey; we expect that upon returning to the monkey for further dissection it will yield additional information which has been overlooked in the sectioned material.

In the cat, by virtue of the presence of an extensive, persistent hypophyseal cleft, the essential independence of the blood supply of the neural process from that of the anterior lobe is assured. The only avenue for communication of the blood vessels of the neural process with other parts of the gland is via the infundibular stalk, and these anastomoses give the impression of being fairly restricted and no larger than capillary size. In the monkey, on the other hand, with the almost complete obliteration of the cleft, leaving only traces of residual lumen, the opportunity for collateral anastomoses between stalk, anterior lobe, and neural lobe is far greater. In our material from the monkey we have traced vascular linkage between anterior lobe and neural lobe via the pars intermedia, as well as numerous anastomoses along the stalk between the various parts of the gland. Nevertheless, in the monkey, too, we regard these internal connections as of a capillary order only, and as subsidiary to the major afferent and efferent systems which, on the whole, are identical in the two forms.

SUMMARY AND CONCLUSIONS

1. The hypophysis of the cat is supplied by superior and inferior hypophyseal arteries. The former send branches to the hypophyseal stalk and anterior lobe, besides giving off a single branch to the infundibular process. The inferior hypophyseal arteries supply the infundibular process and pars intermedia exclusively.

2. The hypophysis of the cat possesses a system of portal venules arising from plexuses of the pars tuberalis and infundibular stalk and passing to the anterior lobe to break up into sinusoids.

3. A set of systemic veins passes from the anterior lobe into the adjacent dural cavernous sinuses; another set of systemic veins passes similarly from the infundibular process and pars intermedia into the nearby cavernous sinuses.

4. There are no venous connections between the hypophysis and the hypothalamic centers situated above the median eminence of the tuber cinereum. There is slight venous drainage from the periphery of the pars tuberalis into the system of basilar pial veins.

5. The blood supply of the hypophysis of the cat is independent, excepting a few capillary anastomoses, from that of the brain proper.

6. The blood supply of the infundibular process and pars intermedia, excepting capillary connections which are numerous, is independent of that of the hypophyseal stalk and anterior lobe.

7. The vascular relations of the pituitaries of monkey and cat are essentially alike, the main difference relating to the median eminence (*eminencia saccularis*) of the tuber cinereum, which is more abundantly vascularized in the monkey than in the cat.

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NEW BOOKS AND PUBLICATIONS

THE MACHINERY OF THE BODY, by Anton J. Carlson and Victor Johnson. 1937. Size, $6\frac{1}{2} \times 9\frac{1}{4}$ inches. Pages xvii + 580. 187 illustrations. 13 tables. List of references. Index. Cloth. Price, \$4.00. The University of Chicago Press, Chicago.

The authors, believing that young college students are genuinely interested in the machinery regulating the animal body, have written a textbook designed to stimulate this innate curiosity. Their success in creating this book, which scientists recommend as being reliable and beginners enjoy as being interesting, can be credited to a comprehensive knowledge of the subject, a close acquaintance with the class to whom it is addressed, and a pleasing perspicuity in the use of words. Physiology is presented as a science built upon experimentation: the experiments of the laboratory and the experiments nature performs in disease. Generalizations are offered tentatively, with the realization that they may be revised by the discovery of new experimental evidence. By this means the authors have avoided those two defects frequently encountered in textbooks: "presentations implying an unwarranted degree of certainty in our knowledge" and "a mere recording of questioned facts and controversial theories."

THE PROPERTIES AND FUNCTIONS OF MEMBRANES, NATURAL AND ARTIFICIAL, A General Discussion. Reprinted from the Transactions of the Faraday Society, April 22 and 23, 1937. Size, $6\frac{1}{2} \times 10$ inches. 241 pages. Cloth. Price, 12/6. The Faraday Society, London.

The sixty-sixth general discussion of The Faraday Society, held in London, England, April 22 and 23, 1937, was devoted to the broad topic of membranes. This volume of the contributed papers, together with the discussions they inspired, serves the useful purpose of presenting an account of a really large subject. Part I describes natural cell membranes. Its chapters deal with the properties of membranes in general, such as structure, permeability, membrane potential, and narcosis; and with special questions relating to red cells, fish gills, egg membranes, and the bioelectrical phenomena of nerve, muscle and skin. Emphasis is laid on the structure of natural membranes, their apparent selectivity in permeability, and the origin of the difference in potential (chemical, osmotic or electrical) which effects and maintains a difference in activity across the membrane. Part II discusses artificial membranes, their preparation, structure, permeability, use, and theories relating to them.

NEW BOOKS AND PUBLICATIONS

PRIMITIVE INTELLIGENCE AND ENVIRONMENT, by S. D. Porteus. 1937. Size, $6 \times 8\frac{1}{2}$ inches. Pages xix + 325. Index. Cloth. Price, \$3.00. The Macmillan Company, New York.

In comparing individuals or races it is hard to discriminate between nature and nurture. This book investigates racial differences in primitive peoples, eliminating as far as is possible all cultural influences. The aborigines of Central Australia and the Bushmen of South Africa were chosen for comparison. The two races are at about the same cultural level although they live in widely separated environments. The Australian deserts are so inhospitable that few living things can survive and the aborigines have a constant struggle with thirst and starvation. The African Bushmen have an easier environment but must compete with wild animals and stronger tribes. Results of tests and observations show that the Australians have more ability in planning and maze solution and are more educable. The Bushmen excel in physical courage and artistic skill. In mastery and use of environment the two races are on a par.

NOTE ON THE EXTERNAL GENITALIA IN THREE FEMALE OLD WORLD PRIMATES

M. F. ASHLEY-MONTAGU

*Departments of Anatomy, The Graduate School and The College of Dentistry,
New York University*

SIX FIGURES

The external genitalia of the Primates, to judge from the examples of which we have some knowledge, present an extraordinary variety of forms. It would be of interest, both from the comparative and morphological viewpoints, to trace the relationships of these differing forms from one species to another, and so, possibly, through every representative group of Primates. Wood Jones, who has made a laudable beginning in this connection, has drawn certain inferences from the appearance of the external genitalia in the Primates examined by him which he thinks throw some light upon the phylogenetic relationships of the latter. It is unfortunately the case, however, that our present knowledge of the structure of the external genitalia of the Primates is characterized more by lacunae than by information, so that attempts to trace relationships between the members of this Order on the basis of such knowledge as we have must, for the present at any rate, remain highly speculative. This is not to say that insufficient attention has been given to the subject, but that there has not been enough material available for study. The Order Primates consists (using Elliot's classification) of more than fifty genera, and somewhere in the vicinity of 600 species, but in spite of the excellent studies of a fairly large number of workers the external genitalia in by far the largest proportion of the genera and species of this Order re-

main undescribed. Wislocki, in the most recent study devoted to this subject has been able to describe and figure the form of the external genitalia in some eleven genera and thirteen species; Pocock has described many more, and other investigators have done their share (see list of references attached to Wislocki's study), yet, as I have said, a majority of Primate species remain undescribed.

Any information, therefore, which may serve to bridge the gaps in our knowledge of this subject are clearly to be welcomed.

In the present communication will be described the external genitalia of three female Old World Primates: an infant gorilla, a young-mature langur, and a mature loris.

The terms of position and direction in the following descriptions are used as if the animal were standing erect in the anatomical position of human anatomy.

GORILLA GORILLA

Infant female, about $2\frac{1}{2}$ years of age. C.-R. length 560 mm. Weight 10 hours after death, 35 pounds. Died in New York City some 5 weeks after arrival from Africa. Cause of death undetermined. No apparent wasting. Examined and drawn 1 week following death and preservation by injection.



Fig.1 Gorilla gorilla. The animal lying on its back with legs separated, showing the normal arrangement of the structures of pudendum muliebre.

In figure 1 the animal is shown lying on its back with legs separated. Some of the hair has been cut away in the region including and adjacent to the perineum the more clearly to display the structures in this region. The perineal hair, it is to be remarked, was only slightly less dense than, though it was quite as long and dark as, the hair of the adjacent surfaces. The pudendum muliebre appears to be situated in a position slightly posterior to the position occupied by the same structure in the human.

Some orienting measurements are:

Bi-ischial diameter (external)	mm. 83
From the posterior margin of the anal orifice to the inferior angular margin of the symphysis immediately above the superior border of the praeputium clitoridis	54
From the anterior margin of the anal orifice to the posterior border of the vulval cleft	13
Length of the vulval cleft to the ventral border of the corpus clitoridis	11

Labia majora

There has always been some question concerning the existence of labia majora in the apes. Wislocki, who has examined the pudenda most carefully in three infant gorillas somewhat younger than the subject here described, observed that

the labia major, as in the chimpanzee and orang, consist in the gorilla also of fatty cushions situated on either side of the medial structures and extending from the symphysis to the perineum. The exact limits of these adipose labial pads are not easily visible, nor can they be readily defined, but they protrude most prominently at the level of the clitoris and the vulval orifice (p. 336).

The average sitting-height of the animals examined by Wislocki was 370 mm., whereas the C.-R. height in the present subject is 560 mm. Our animal is, therefore, somewhat older, and this may account for the difference in the appearance of the labia majora as between this and the animals examined by Wislocki. The latter investigator has pointed out that in

the great apes during early life the labia majora are extensive and bear a striking similarity to the human, but that in ontogenetic development they undergo a marked reduction "in some individuals amounting to practically total disappearance" (p. 339, n. 2). It is quite probable that in our subject the labia majora exhibit one of the stages in this ontogenetic reduction. The animal is, therefore, not without a peculiar interest.

The labia majora are represented by two narrow cushions of skin situated immediately lateral to the praeputium clitoridis, and laterally extend from a level a little above the praeputium posteriorly and medially, but always lateral to the praeputium, to meet the labia minora at the external postero-lateral angle of the praeputium. These folds do not meet above the praeputium at the symphysis, and, as may be seen in figures 1 and 2, they do not appear to be in any way represented in the region of the vulval orifice; hence, anteriorly it is difficult to recognize a definite mons veneris; nor is an anterior commissure of human anatomy recognizable since the labia majora do not meet anteriorly. Although the labia majora do not meet posteriorly, a posterior commissure may be recognized, but this is formed by the junction of the labia minora. The absence of a mons veneris is apparently characteristic of the adult as well as of the infant gorilla, for Gerhardt, who described the external genitalia in an animal which he estimated to be between 11 and 12 years, remarks that "Von einer nennenswerten Fetteinlagerung, die mit einem Mons veneris vergleichbar wäre, ist nichts zu bemerken" (p. 637). Wislocki found no evidence of a mons veneris in the infant gorillas examined by him, although he says of the great apes generally, that,

especially during early life, when the labia majora are extensive and bear a striking similarity to the human, that portion of the labia majora extending forward onto the symphysis can well be homologized with the human mons veneris. Since, however, the labia majora undergo marked ontogenetic reduction in the apes, in some individuals amounting to practically total disappearance, a mons veneris is at best less easily distinguishable than in the human (p. 339, n. 2).

In the present animal the labia majora are accurately described as lying in a plane anterior and superior to the labia minora, but not lateral to them. This is, of course, distinctly but not fundamentally different from the human condition. The length of each labium is 25 mm., and the breadth 5 mm. The deep black pigmented surfaces show a sparse growth of

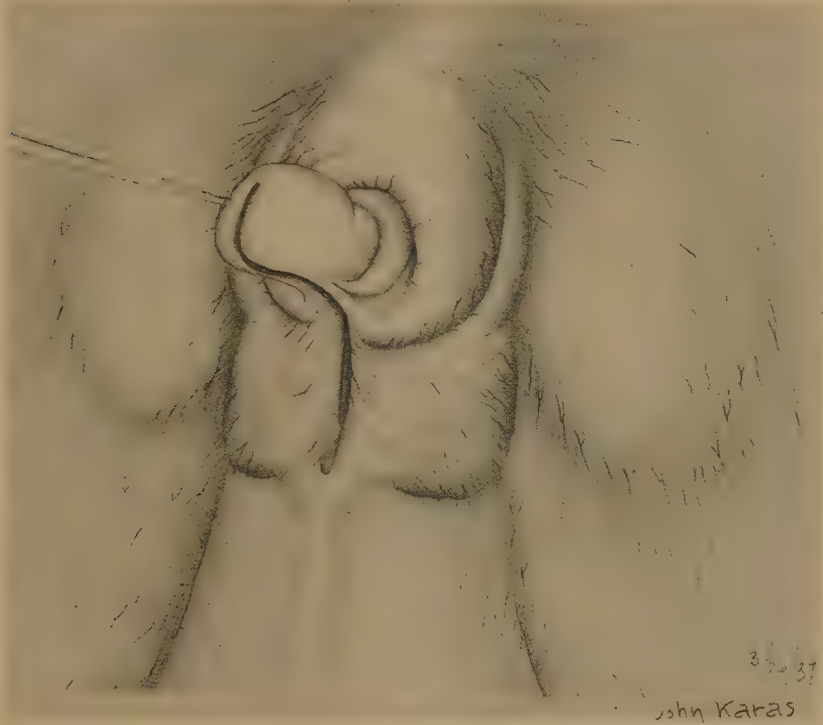


Fig. 2 Gorilla gorilla. A closer view of the same subject with the clitoris extracted.

hair, are pitted at regular intervals, and no doubt contain sebaceous as well as sweat glands.

Lateral to the labia majora are situated two structures which do not appear to have been previously noted in this region in any of the Primates. These structures are so prominent that they were at first taken to represent the labia

majora, to which, possibly, they may bear some morphologic relation. These structures are represented by two fatty cushion-like elevations, each measuring 32 mm. in length and 14 mm. in breadth, and situated immediately lateral to the lateral pudendal groove bordering the labia majora upon the inner superior-most aspect of the thigh. They commence lateral to the labia majora at a level a little above the superior border of the praeputium and extend posteriorly to a level lateral to and slightly anterior to the posterior borders of the labia minora. These lateral structures depend caudally, below the level of the adjacent skin into which they gradually merge, to a maximum distance of some 12 mm. When the thighs are approximated to one another these structures appear even more prominent than they do in the figures, which show the animal with its legs spread apart, and might easily be mistaken for labia majora. That these structures in the course of evolution may have become incorporated in the true labia majora is a possibility, a possibility, however, which for the present must remain no more than a speculation. It is also possible that these adipose cushions may represent the lateral migration of tissue earlier devoted to the true labia majora; whatever their significance may be, they would appear to be closely related to the labia majora.

It is of interest here to note that the inner aspects of the thighs in this region are, in our subject, strikingly undeveloped and flattish, unlike the same regions in the human female where the inner aspects of the thighs are normally well developed, rounded, and encroach upon the labia majora. It is possible that the peculiar development of the human thigh in this region may in some way be associated with the development of the characteristic form of the human labia majora.

Labia minora and clitoris

The labia minora are represented by quadrilaterally shaped fatty cushions situated posterior to the praeputium on each side of the vulval cleft ("Centrally, surrounding a short pudendal cleft, there are conspicuous rosette-like folds, the

labia minora." Wislocki, p. 336). Laterally the labia are bounded by the inner aspects of the upper thigh, posteriorly by the floor of the perineum, medially by the vulval cleft or vestibule, antero-laterally by the labia majora from which they are separated by a slight depression at the level of the postero-lateral praeputial angle. Medially the labia continue into the praeputium from which also they are separated by a slight depression. The length of each labium is 11 mm., and the breadth 12 mm. The labia minora are, therefore, slightly broader than they are long. Extending from the postero-medial border of the vulval orifice to the antero-medial border of the anal orifice there is a well-marked perineal raphe. The size of the anal orifice in this animal, which appears rather large, is due to the fact that some time before death it had suffered a prolapse of the rectum. The length of the perineal raphe is 13 mm. The elevation, or dependence, of the labia below the level of the adjacent tissues is 5 mm. The external surfaces of the labia are pigmented black and are completely hairless; the caudal surfaces are a little puckered and pitted, suggesting the presence of sebaceous glands. There is, however, practically no puckering at the medial lips bordering upon the vulval cleft. Medially the labia fit together so neatly that the orifice is quite obliterated; in the active living animal with a full growth of perineal hair it would be quite a matter of some difficulty to distinguish its presence.¹ The length of the vulval cleft from the anterior margin of the perineal raphe to the base of the corpus clitoridis is 11 mm. The vulval surfaces of the labia, or lumen, are quite unpigmented and present a glistening white appearance which stands in marked contrast to the adjacent deeply pigmented external surfaces.

After a very careful exploration, repeatedly made, no traces of a hymen or anything corresponding to such a structure could be found. The vestibule is uninterrupted by the presence of any structure within it from the perineal border to the base of the corpus clitoridis.

¹ The authorities of zoological gardens, and even dealers, have been known to purchase male gorillas which have eventually proved to be quite normal females!

Since posteriorly the labia minora meet in the midline at the anterior border of the perineal raphe, but without establishing any relation with the labia majora which, in the gorilla are not present in this region, it would be quite possible to speak of the existence of a posterior commissure in this animal, but it would be necessary to note that it is formed by the labia minora alone. There is no frenulum labiorum and nothing corresponding to a vestibular fossa anteriorly.

As we have already noted, antero-laterally the labia minora continue into the labia majora. Medially the labia minora continue into the praeputium clitoridis. Before describing the relations of the labia minora to the clitoris it will be necessary here to give a brief description of that organ.

The clitoris is a well-developed organ possessing a body and a glans, the respective dimensions being as follows:

	<i>mm.</i>
Length of corpus clitoridis	9
Breadth of corpus clitoridis	5
Height of corpus clitoridis (dorso-ventral)	7
Length of glans clitoridis	5
Breadth of glans clitoridis	6

A peculiarity of the clitoris of the gorilla, which it shares with some other groups of Primates, is that both the body and the glans are practically divided by a ventral cleft which extends throughout the whole length of the organ and is continuous with the vulval or urogenital cleft postero-inferiorly. As in the case of the labia minora the external surfaces of the clitoris are deeply pigmented, but the internal surfaces of the cleft being continuous with the lumen of the urogenital orifice are of the same conspicuous whiteness.

Superiorly the medial borders of the labia minora produce a fold of tissue which passes into the base of the glans to merge gradually into the anterior medial margin of the incisura clitoridis; this is the frenulum of the praeputium clitoridis. Laterally this fold gradually becomes enlarged and passes superiorly over the clitoris as a substantial roll of skin to form the praeputium major. In the relaxed condition,

shown in figure 1, the clitoris is, as it were, sheathed by the praeputium major, its anterior aspect at the glans alone being visible as it lies within the praeputium major. The diameters of the praeputium major are:

	<i>mm.</i>
Transverse diameter	26
Antero-posterior diameter	19

The margins of the praeputium major at the glans are somewhat puckered and exhibit the presence of but a few short hairs; above these margins, however, there is a somewhat stronger growth of hair.

Wislocki has stated that a well-developed praeputium in the infant gorilla reported by him was without a frenulum (pp. 335-336). In the present animal a frenulum was not only present, but was also well developed, as may be seen in figure 2.

Originating from the frenulum of the praeputium major, a little lateral and superior to the terminal portion of that structure at the base of the glans, there is another and smaller roll of tissue which arches over the body of the clitoris a little distance behind the glans under cover of the praeputium major with which it is continuous; this is the praeputium minor and its frenulum (fig. 2). The praeputia major and minor are both pigmented a deep black.

The embryological origin of the praeputium in the human species has been tentatively described by Spaulding as arising from a pair of swellings lying on each side of the urethral opening, and first recognizable in embryos of about 65 mm. C.-R. length.

Gradually these swellings fuse together over the dorsum of the shaft to form a flattened ridge of skin whose distal margin has enveloped the proximal margin (corona glandis) of the glans (embryos 75 mm. C R length). Subsequent growth results in the progressive inclosure of the glans by this distally migrating fold of skin until at about 100 mm. C R length the originally naked glans is entirely covered (p. 85, compare also p. 83).

This description suggests, and the relations of the relevant structure in the gorilla here described—as well as my own examination of these structures in living and deceased human females—attest, that the praeputium actually originates from the superior limits of the urethral folds. Certainly in the adult human female both the frenulae and the praeputium appear to be best explained as arising from the continuing terminal portions of the labia minora (urethral folds).

In connection with the clitoris and its development it may be of interest here to note that at no stage in the development of the human female is the glans clitoridis ventrally cleft (Spaulding, p. 72), the urogenital cleft being limited to the corpus clitoridis alone; the closure of the cleft taking place at some time after the 100 C.-R. stage. In the human male, however, the urogenital cleft extends through the body of the phallus into the glans as far as the epithelial tag. In fact, a 32-mm. C.-R. human male embryo normally presents a phallic groove very like that of the female gorilla shown in figure 2. In the adult human male the glans exhibits the remains of this groove in the hiatus extending from the meatus to the coronary sulcus ventrally. It is of interest that the remains of this cleft is in the adult male gorilla exhibited even more markedly than in man, the glans according to Duvernoy (quoted by Sonntag) appearing to be cleft almost in two portions.

I have endeavored to find traces of a ventral cleft in the clitoris of the human female, but in the small number of twelve subjects which I have had an opportunity of examining (three dead infants, four dead and five living adults—all Whites) I failed to find any traces of such a cleft. A search through the literature failed to reveal any mention of such a condition in the human. It is, however, not difficult to conceive of the manner in which the human type of clitoris could be derived from a gorilla-like clitoris, all that would be necessary would be a reduction of the clitoris to the size exhibited by that of the human female, followed, possibly, by a fusion superiorly of the embryonic urethral folds. It may, incidentally, be re-

marked that as far as I am aware the human clitoris is smaller than that of any other known Primate.

THE PURPLE-FACED LANGUR (*Kasi vetulus nestor*)

Young mature female. Colombo, Ceylon, C.-R. length 330 mm. Wild specimen. Examined and drawn about 6 months after death and continuous immersion in formalin.

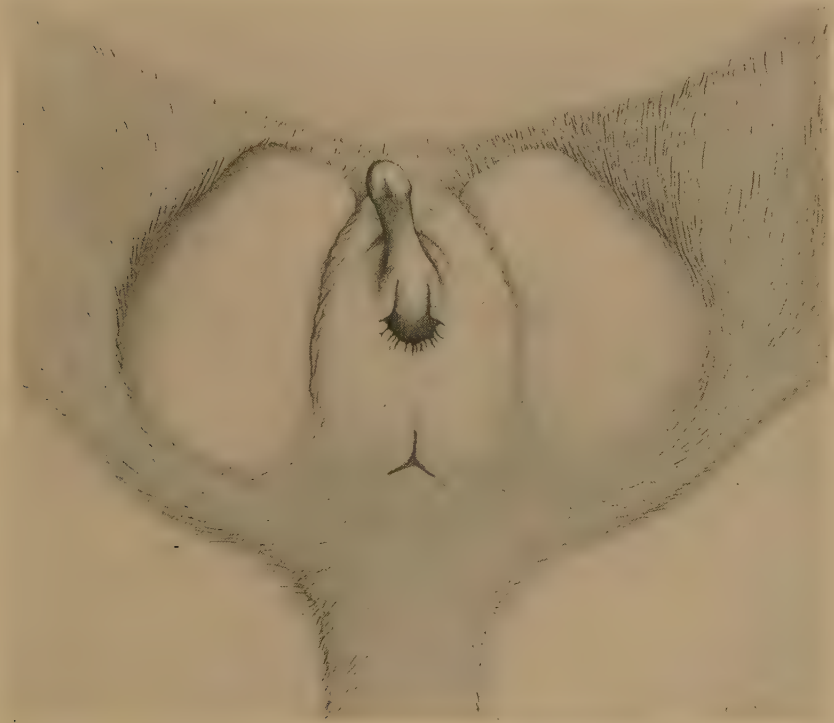


Fig. 3 *Kasi vetulus nestor*. The animal lying on its back with legs separated, and tail depending.

In figure 3 the animal is shown lying on its back with its tail depending. The lateral bare areas seen in the figure are the extensive naturally formed ischial callosities which are found more or less well developed in practically all Old World monkeys, and are almost exclusively limited to them

as a group. The hair has been left intact in the regions figured, but in the region of the perineum the hair is not quite as strongly developed as it is elsewhere on the body. Some relevant measurements are:

	<i>mm.</i>
Bi-ischial diameter (internal)	13
From the posterior margin of the anal orifice to the inferior angular margin of the symphysis	25
From the anterior margin of the anal orifice to the posterior border of the vulval cleft	7
Length of the vulval cleft from the posterior commissure to the corpus clitoridis	4

Labia majora

No evidences of structures corresponding to labia majora were present, there are, therefore, no labia majora in this animal (fig. 3).

Labia minora and clitoris

The labia minora are not elevated beyond the level of the adjacent skin and are represented by two simple folds of skin which posteriorly are continuous with the puckered folds lying at the posterior border of the vulval orifice, and which, presumably, are to be regarded as parts of the labia minora. Anteriorly the labia are not puckered but present a smooth appearance, and terminate upon the ventrolateral aspects of the clitoris, at its basilar portion, as the frenulae of that organ (fig. 3). A scarcely perceptible fold of skin continuing antero-laterally from the latter and arching over the clitoris is probably to be regarded as a weakly developed praeputial element. Osman Hill has, however, been unable to recognize a praeputium in a related variety of this species (*Pithecus vetulus*, Osman Hill, p. 50 a). The length of each labium is 4 mm. The posterior border of the vaginal orifice is relatively rather broad, the antero-posteriorly elongated vulval cleft narrowing anteriorly toward the clitoris. Short hairs are plentifully distributed both upon the labia and the fold of skin which I have taken to represent the praeputial element.

The clitoris, which is extra-labial, is very well developed, consisting of a body and glans, the dimensions of these are:

	<i>mm.</i>
Length of corpus clitoridis	8
Breadth of corpus clitoridis	3
Height of corpus clitoridis	3
Length of glans clitoridis	4
Breadth of glans clitoridis	4

The whole of the clitoris is unpigmented. A slight corona glandis is observable on the dorsal and dorso-lateral aspects of the organ, but not upon the ventral aspect. There is a slight notch on the ventral aspect of the glans which in the present specimen is not easily distinguishable. I was unable to discover any evidences of the ventral grooving of the corpus clitoridis which Osman Hill has described in the clitoris of *Pithecus vetulus* (p. 50). There is no tunneling of the clitoris. A perineal raphe was not observable, though it was carefully looked for. No evidences of the existence of a hymen were to be found.

THE SLOW LORIS (*Loris tardigradus*)

Mature female. Ceylon. C.-R. length, 191 mm. Wild specimen. Examined and drawn about 6 months after death and continuous immersion in formalin.

In figure 4 the lower abdominal and perineal facies of the animal are shown. The praeputium and clitoris look rather large, but in reality they are, in relation to the C.-R. length of the animal, half the size of the male structures present in the gorilla earlier described, but somewhat more than twice the relative size of the same structures in the langur.

	<i>mm.</i>
Bi-ischial diameter (external)	13
From the opsterior margin of the anal orifice to the inferior angular margin of the symphysis	14
From the anterior margin of the anal orifice to the posterior border of the vulval cleft	8
Length of the vulval cleft from the posterior commissure to the corpus clitoridis	4

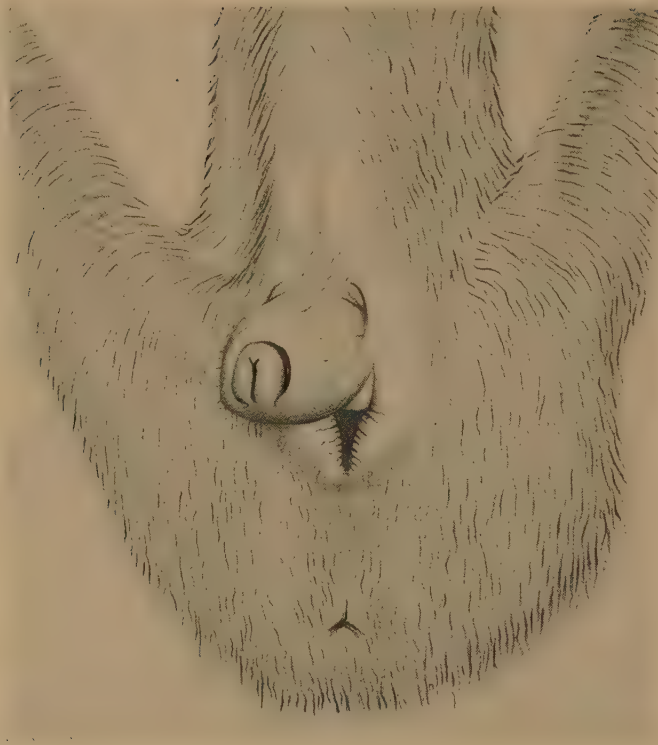


Fig.4 *Loris tardigradus*. The animal leaning slightly backward with legs displaced upward and laterally showing the normal arrangement of the structures of the pudendum muliebre.

Labia majora

There are no labia majora.

Labia minora and clitoris

The labia minora, as in the langur, are not elevated beyond the level of the adjacent skin and are represented by two simple folds which posteriorly are continuous with the puckered folds of skin which lie at the posterior apical portion of the vulval orifice. The anterior unpuckered portions of the labia minora terminate antero-medially upon the ventro-lateral aspects of the base of the clitoris. These

structures, the labia and the base of the clitoris, give form to the vulval orifice, which is triangular shaped, with its base at the ventral aspect of the clitoris and its apex at the posterior commissure. The anterior terminal portions of the labia minora may be regarded as the frenulae of the clitoris, and are clearly displayed in figure 5. Laterally and anteriorly the labia are continuous with the skin which overlies the clitoris. This skin presents certain extremely interesting features. It is, in the first place, continuous with the skin of the lower abdomen, and dorsally and laterally projects forward to cover the clitoris. The fact that this skin merges into the clitoris ventro-laterally results in an appearance as if the clitoris were completely ensheathed by this skin, but this is not quite so. Anteriorly and ventro-laterally this skin passes medially and dorsally on each side to form the anterior limit of the clitoris, and thus give rise to a structure which resembles the higher Primate glans clitoridis (figs. 4 and 5), and which, if it is not homologous with, is at any rate, analogous to that structure in the higher Primates. The glans presents a dorso-ventrally elongated orifice, the skin on either side of which being marked by the presence of a fair number of short hairs. The skin on the dorsal and lateral aspects of the clitoris is covered by a good growth of short hairs, but the ventral aspect of the clitoris, with which this skin merges, is completely devoid of hair, except at the glans. The clitoris is some 6 mm. in length and 3 mm. in breadth, the breadth of the glans is some 4 mm.

When the skin covering the clitoris is retracted, as in figure 6, a fold of skin originating from the small frenulum clitoridis is seen arching over the body of the clitoris—this we take to be the *praeputium minor*, the retracted fold of skin above the latter which covers the clitoris, we take to represent the *praeputium major*. The relations of the various structures are here better understood if the clitoris is imagined to be greatly reduced in size, and ventrally cleft. If the reader will create the image for himself he will then readily see the homologous relations as between this creature's pudenda and, let us say, those of the gorilla here described.

The following observations by Osman Hill on the external genitalia of the loris (*Loris tardigradus*) will complete my own. Owing to the condition of the specimen in my possession I have been unable to determine the presence of those structures referred to by this investigator in the following passages.

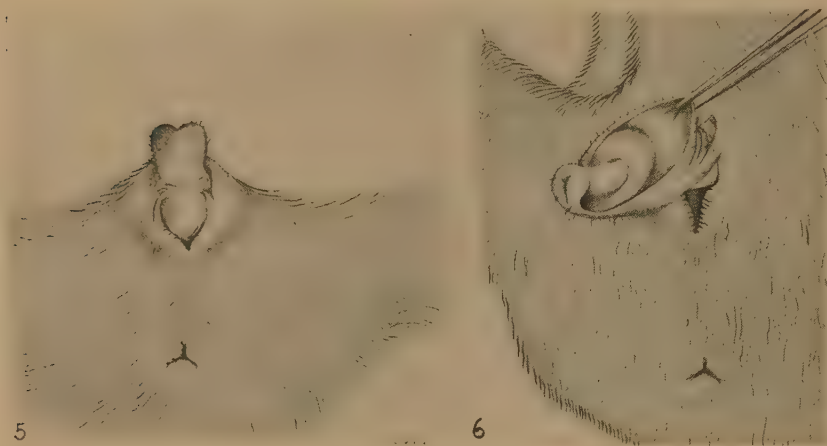


Fig. 5 *Loris tardigradus*. The animal lying on its back showing a ventral view of the clitoris and the frenulum derived on each side from the labia monora.

Fig. 6 *Loris tardigradus*. The animal in a sitting position leaning slightly backward. The praeputium major is retracted to show the praeputium minor, the corpus clitoridis, and the form of the glans clitoridis.

The glans is bifid, ending in two swollen protuberances lying side by side and separated by a deep cleft. In the bottom of this cleft and towards the dorsal side lies the external opening of the urethra, which has traveled beneath the hairless tract of skin on the corpus clitoridis to reach this position. In *Loris*, therefore we have the complete degree of tunnelling of the clitoris by the urethra. . . . At the tip of each of the swellings on the end of the clitoris is a glandular pocket, one on each side of the urethral opening. Each of these is a vertically-elongated structure with the lateral lips in contact Dorsal to the two pockets in *Loris* there is a tuft of long hairs on each side of the glans (p. 103b).

I wish here to record my thanks to Prof. W. C. Osman Hill of The Medical College, Colombo, Ceylon, who very generously presented me with the langur and the loris described in these notes. To Dr. Endre K. Brunner of the Department of Obstetrics, New York University Medical College, I am indebted for his kindness in making possible the examination of the external genitalia in living subjects.

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For additional references see the paper by Wislocki.

PRELIMINARY STUDIES OF HEREDITARY VARIATION IN THE AXIAL SKELETON OF THE RABBIT

PAUL B. SAWIN

Brown University

THREE FIGURES

One of the best known, though less well-understood variations in the vertebrate skeleton is that in which one of the component parts of the axial skeleton assumes the morphological appearance and function of its neighbor either immediately preceding or immediately following it. Such variations were designated homeotic by Bateson (1894), in distinction from meristic variations characterized by changes in total number of component parts. They have been studied intensively by numerous authors in a wide variety of animals, including man. So far as I am aware, however, only two attempts have been made to determine definitely the inheritance of such variations, although they are considered of singular importance in the evolution of man and of many other forms.

Early investigators of homeotic variations have been divided between two schools—the larger, headed by Rosenberg who believed in the fixity of numerical homologues in the vertebrae, and that homeotic variations are to be explained either as due to the assumption of lumbar characteristics by a thoracic vertebra, or to the shifting of the pelvis in both ontogeny and phylogeny. Members of the other group, headed by Welcher, have contended that numerical identity cannot be determined, but that variations occur by the intercalation or excalation of parts with respect to given points such as the skull or the auricular surface of the sacrum. Attempts have

been made by DuToit, Bateson, Kingsley, and others to reconcile these two theories, but Danforth ('30) as the result of further study from the morphological standpoint is inclined to discard both ideas and suggests the necessity of considering other fields, such as genetics and embryology, for the causal relations of such variation.

In the genetical field, Promptoff ('28) has studied variation in the lumbo-sacral region of domestic breeds of poultry and has explained the presence of three, four or five vertebrae in the dorso-sacrum on a definite factorial basis of three genetic factors. Two of these, one of which is dominant and the other recessive, are in his view responsible for the development of the five-dorsal type versus the four-dorsal type. The other hypothetical factor is a dominant which when present produces the three-dorsal type. But this variation was difficult to study in living animals and so Promptoff was not able by random selection to secure sufficient inbred matings of normal and abnormal types to constitute a critical test of the validity of the triple-factor hypothesis which he offers. The use of three genetic factors in interpreting a variation, as has been pointed out by Wright ('34), permits a wide choice of formulae to account for observed ratios and although such choice is capable of accounting for the variation in genetic terms, it does not constitute proof that the formulae are correct.

Kuhne ('32), as the result of extensive pedigree studies in man and other animals arrived at the general conclusion that homeotic variations can be explained on the basis of single unit factors which do not affect a single vertebral unit, but produce a 'tendency' for homeosis in a given region. Frede ('32) has shown that in the rat, nerves and muscles as well as vertebrae of a given region are affected, and Fischer ('33) thinks that such variations are engendered by genes which retard or accelerate growth in a given region and therefore are subject to considerable variation in their manifestation. This is a principle previously endorsed by Fisher ('30) who supposes that "the actual number (of vertebrae) exhibited is but the somatic expression, to the nearest whole number of an

underlying physiological variation influenced like a simple measurement by both environmental and genetic causes.”

Because of the complexity and particularly the continuity displayed by this sort of variation and the difficulties involved in determining phenotypes, it is not the most satisfactory genetic material to work with but, because of the knowledge to be gained of a developmental problem which has so long been debated by morphologists and paleontologists, it is an extremely interesting and important character.

My attention was first directed to the problem by the observation in rabbits used for class dissection, of the frequent occurrence of ‘gorilla ribs,’ i.e., supernumerary ribs borne on lumbar vertebrae. These rabbits were descended from animals used in genetic experiments, which made possible an attack upon the problem of inheritance of the abnormality. Examination of the literature showed that the character had been observed in the rabbit as early as 1566 by Coiter. It is known also to occur sporadically in most other vertebrates.

For the past 5 years this variation has been under continuous observation in our rabbit colony and special matings have been made to discover, if possible, whether it is inherited and if so, how. Over 3000 individuals of known parentage have been examined either by x-rays or by dissection.

TECHNIQUE USED IN DETERMINING BIOTYPES

The rabbit is excellent material in which to study vertebral variations for several reasons. Clear x-ray pictures¹ are easily obtainable in which the component parts do not overlap enabling the accurate identification of living phenotypes. The vertebrae and ribs are well established at birth, and newborn

¹ The author is greatly indebted to Dr. Philip Batchelder for many valuable suggestions and particularly for the generous use of his x-ray equipment, and for the unfailing interest and cooperation of his assistant, Mrs. Bienvenue, who has taken the x-ray pictures without which these studies would have been greatly handicapped, if not impossible. My thanks are also due to Dr. Sheldon Reed for his assistance in recording data during the summer of 1932, and especially to Dr. W. E. Castle for his interest and council, and in whose laboratory a major portion of these animals has been housed during the several years that the study has been in progress.

rabbits are of a convenient size for easy dissection, making possible the accumulation of data with reasonable rapidity. Skeletons at this age may be easily and quickly cleaned by gently boiling the carcass for several minutes, which loosens the muscle and connective tissue, and such specimens may be dried or stored in formalin. Such has been the general procedure. Occasional specimens, the classification of which was in doubt, or which were somewhat fragile, have been cleared by the Spalteholtz method used by Strong ('25) and stained with alizarin.

TYPES OF VARIATION IN THE AXIAL SKELETON

Authors of laboratory manuals differ as to the normal formula for the axial skeleton of the rabbit, which fact indicates the existence of some variability. Bensley ('31) gives the normal formula as 7/12/7/4/16, the numerals representing in order the number of cervical, thoracic, lumbar, sacral, and caudal vertebrae. Crabb ('31) states the normal number of thoracic vertebrae to be twelve but notes the occasional occurrence of thirteen pairs of ribs. His list of skeletal components includes eight lumbar, three sacral, and twenty caudal vertebrae.

As in mammals generally, variation of the number of cervical vertebrae in the rabbit is comparatively rare. Only two exceptional cases have been observed in these studies. Both of these occurred in the progeny of the same parents and both were deficient in rib number and had eight cervical vertebrae. The mother was but remotely related to any of the other family lines. Since they represent an extreme type of variation, out of line both morphologically and genealogically with the data to be discussed here, they will not be taken into account further.

Sacral vertebrae are by definition those which are coalesced in the adult to form a composite structure, the os sacrum, which supports the pelvic girdle at the facies auricularis. Inasmuch as authorities differ as to the number of elements involved, a variation which may easily be due to age differ-

ences of the animals upon which observations are based, and also since in this study most individuals have been examined at an early age when there is little or no fusion manifest, it was found necessary to set some arbitrary number as that belonging to the sacrum. Three has been used uniformly in this study.

Caudal vertebrae are the most variable group and are of importance primarily in studies of meristic variation which will not be discussed here.

With the exception already noted, the animals studied in this investigation all agree in having seven cervical vertebrae; three vertebrae regularly enter into the constitution of the sacrum, and the caudal vertebrae vary from fourteen to nineteen, with about sixteen as a modal number. No further notice need be taken of these regions of the axial skeleton.

As regards thorax and abdomen, the animals fall into four types, though intergrades occur between them (fig. 1). The type most commonly encountered, among rabbits of small to medium size, has twelve thoracic and seven lumbar vertebrae. This we may designate type I, the normal. Type II differs from type I in having a thirteenth pair of ribs borne on the metamorphosed first lumbar vertebra. Thereby the number of true lumbar vertebrae is reduced to six. The formula of this type accordingly is 13/6 instead of 12/7, as in type I, but the total number of presacral vertebrae is the same in both types (twenty-six). In type III, the twenty-seventh vertebra is not attached to the sacral complex and appears as a lumbar vertebra without any change occurring in the thorax. The formula of this type accordingly is 12/8. Type IV combines the departures from normality found in types II and III. Its formula accordingly is 13/7. The total number of presacral vertebrae is the same as in type III, viz., twenty-seven, but there is one more rib-bearing vertebra.

The frequency with which these types appear in the general population is probably indicated by table 1, in which is shown a classification of the first 489 animals examined. They are shown in such a manner as to compare the homeotic variations

with the meristic variations as indicated by the number of caudal vertebrae. Selection was made at random from six races of rabbits being bred at the Bussey Institution in 1932 and the results seem to indicate no correlation between the two sorts of variation. These and two additional races since

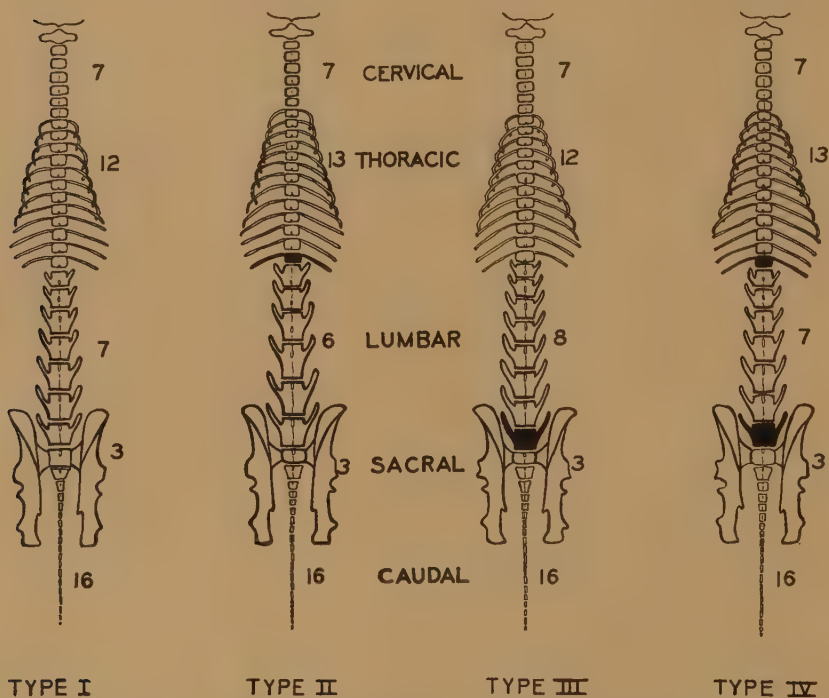


Fig.1 Types of variation with respect to number of ribs and of presacral vertebrae.

examined may be divided into three groups according to size: small, medium and large. The small races tend to express the normal type in a true breeding condition; the medium and large races display the abnormal types. In none of these families where sufficiently large numbers of progeny have been examined has strict homozygosity been observed. In one family there is strict normality in the thoraco-lumbar region, but variation in the lumbo-sacral region though inconspicuous is nevertheless present and this is of considerable significance as data from crosses described below will show.

While these four formulae are very convenient and have been used for the basis of this study, the results indicate that in future studies greater emphasis may have to be placed upon the manifestations of the individual homeoses and less upon formulae of this type. Lest the reader develop the misconception of complete discontinuity between these types it should be pointed out that all degrees of variation have been found to exist between them and any attempt to classify appears to be an arbitrary matter.

TABLE 1

Showing the frequency of the several vertebral type formulae as they occur in the general rabbit population and as they are associated with varying numbers of caudal vertebrae (see text)

THORACO- LUMBAR RATIO	NUMBER OF CAUDAL VERTEBRAE						Total
	14	15	16	17	18	19	
12-7	3	13	199	97	13	1	326
13-6		5	54	39	7	1	106
12-8			3				3
13-7		3	34	9	7		53
13-8			1				1
Total	3	21	291	145	27	2	489

Throughout these experiments it has been customary to record as thirteen-ribbed animals all which showed the character in any degree, varying all the way from completely developed ribs to mere stubs or slightly modified lumbar transverse processes. Frequently asymmetry has been noted and such conditions both in rib and sacral attachment have always been recorded either by description or diagram, and will be the basis of a later contribution. In the more recently recorded data an effort has been made to evaluate the degree of rib expression attained by the progeny of individual mothers. This has been accomplished by grading the development of the extra rib on each side on a scale ranging from 0 to 4, grade 4 designating a fully developed rib. The individual score has then been calculated by taking the average of the grades assigned to the right and left components. Matings between

rabbits of these different types have been studied in three different families and the results are tabulated for ready comparison in tables 2 to 4.

INHERITANCE STUDIES

In table 2 is recorded the first set of experimental matings. A single mating was made between parents both of type I. Five of the young were also of type I but a sixth individual was of type IV, displaying both manifestations of abnormality. This makes it clear that normals are not true-breeding in this family. Type I mated with type IV produces both

TABLE 2
Classification of the young produced by mating within family 1

PARENTS	OFFSPRING					PER CENT HAVING AN EXTRA RIBS	PER CENT HAVING AN EXTRA LUMBAR VERTEBRA	PER CENT HAVING BOTH
	I	II	III	IV	Total			
I $\times$ I	5	0	0	1	6			
I $\times$ IV	48	15	5	36	104	49.0	39.4	34.6
III $\times$ IV	12	2	2	11	27	48.1	48.1	40.7
IV $\times$ IV	12	12	4	31	59	72.8	59.3	52.5
Totals	77	29	11	79	196	55.1	45.9	40.3

those types as well as a lesser number of the intermediate types (II and III). Type IV animals mated together produce chiefly animals of that same extreme type but with lesser numbers of other types, including normals (type I). It is clear from the foregoing that neither extreme condition (type I or type IV) breeds true. Each type is capable of producing all the others, which eliminates the possibility of a simple mendelian explanation.

Family 2

Family 2 was studied extensively and may best be discussed as three sub-families bred somewhat differently. The young classified in table 3a were produced by parents selected for extra ribs only (type II) or by such animals mated with nor-

mal sibs or with unrelated normals (type I), or finally by normals of extra rib ancestry mated together. Type II parents produce a majority of young like themselves in character, as also do type I parents. Matings between the two types produce both sorts in nearly equal proportions, though many extra-ribbed individuals have also the extra lumbar vertebra and thus become type IV animals. Even normals mated together ($I \times I$) produce type IV as well as type II young.

In general, in this family there does not appear to be conformity of the vertebral types as outlined above to any simple mendelian interpretation. However, if the presence or absence of the extra-ribbed condition is considered alone, there

TABLE 3a

Classification of the young produced by matings of normal and extra-ribbed parents; family 2A

PARENTS	OFFSPRING					PER CENT HAVING EXTRA RIBS	PER CENT HAVING AN EXTRA LUMBÆ VERTEBRA	PER CENT HAVING BOTH	DEGREE OF EXPRES- SION OF EXTRA RIBS
	I	II	III	IV	Total				
$I \times I$	27	6	0	6	39	30.7	1.5	1.5	58.3
$I \times II$	138	105	0	44	287	51.9	15.3	15.3	62.3
$II \times II$	37	85	0	19	141	73.7	13.4	13.4	74.5
Totals	202	196	0	69	467	56.7	14.7	14.7	65.0

is a close approximation to the number of normal and abnormal individuals to be expected in each of the three types of mating, on the basis of a single dominant gene causing the production of extra ribs. The type II parents used here were not exclusively of type II parentage.

The presence of type II and type IV progeny resulting from type I parents suggests the presence of modifying genes, further evidence of which will be discussed in connection with family 2C.

The parents in table 3b were chiefly normals of normal parentage (type I) and were, for the most part, mated inter se, but a few matings were made between a type I male and his type II daughters. Matings between type I parents produced 321 type I offspring (80% of the total), but there were

also eighty-one individuals which were classified as type II (having an extra pair of ribs) and one individual had also an extra lumbar vertebra and was thus of type IV. Matings between type I and type II individuals produced a majority of type II or type IV offspring, though seventeen individuals

TABLE 3b
Classification of the young produced by matings in family 2B

PARENTS	OFFSPRING					PER CENT HAVING EXTRA RIBS	PER CENT HAVING AN EXTRA LUMBAR VERTEBRA	PER CENT HAVING BOTH	DEGREE OF EXPRES- SION OF EXTRA RIBS
	I	II	III	IV	Total				
I $\times$ I	321	81	0	1	403	20.3	0.2	0.2	45.2
I $\times$ II	17	24	0	3	44	63.6	6.8	6.8	64.7
Totals	338	105	0	4	447	23.4	0.9	0.9	54.9

(38%) were of type I. This family in general manifests a lower percentage of abnormal individuals, and the abnormality, where present, is less well expressed than in family 2A, as is shown by the last four columns of each table. This difference may be regarded as a consequence of selection for the normal type (I).

Table 3c includes matings between pairs of what were originally considered to be type I parents and between pairs of type IV individuals as well as between mixed combinations. If the first mating is considered a I $\times$ I mating, it at once is apparent that the mating is not genetically the same as the

TABLE 3c
Classification of the young produced by matings within family 2C

PARENTS	OFFSPRING					PER CENT HAVING EXTRA RIBS	PER CENT HAVING AN EXTRA LUMBAR VERTEBRA	PER CENT HAVING BOTH	DEGREE OF EXPRES- SION OF EXTRA RIBS
	I	II	III	IV	Total				
I $\times$ I	21	21	0	11	53	60.3	20.7	20.7	76
I $\times$ II	4	0	0	2	6				
I $\times$ IV	14	22	0	10	46	69.5	21.7	21.7	74.5
II $\times$ IV	8	17	0	40	65	87.7	61.5	61.5	83
IV $\times$ IV	0	12	1	60	73	98.7	80.6	80.5	86.3
Totals	47	72	1	123	243	80.2	51.0	50.6	79.9

corresponding mating in families 2A and 2B since a much higher proportion of abnormals results. This is better shown in column 7, where the obtained per cent of extra ribs is shown to be 60.3% in 2C as compared with 30.7 and 20.3% in the other two families. It is also reflected in the high degree of extra rib development (see the last column), 79% in 2C as compared with 65.0 and 54.9% in the others.

There is also a higher proportion of abnormals among the progeny of the other matings listed in table 3c as compared with those of tables 3a and 3b. In addition to the thoracolumbar difference there is also a larger per cent of individuals

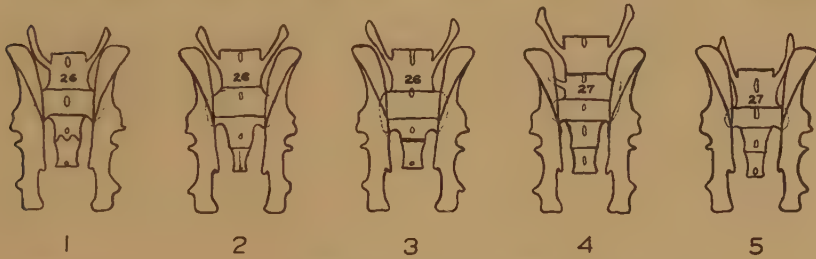


Fig. 2 Variations in the attachment of the pelvis in family 2C may be arbitrarily classified into the above types, according to the manner in which they involve the twenty-seventh or the twenty-eighth vertebra or both. 1 is the normal attachment to the twenty-seventh vertebra alone and 2 to 5 are various degrees of more posterior union.

having an extra presacral vertebra (column 8). The full import of this fact resulted from a more detailed analysis of the lumbo-sacral region.

In an effort to find an explanation for the more frequent development of abnormality in family 2C and for its stronger expression, a reexamination was made of the x-ray pictures and dissections of the individuals in this family. It was found that in many individuals classed as type I (normals) both among the parents and their offspring, there existed a tendency for the chief attachment of the sacrum to be shifted backward from the twenty-seventh to the twenty-eighth vertebra, as indicated in figure 2. All gradations were found to exist between the situations indicated in 1 and 5 of figure 3.

The mother from which all individuals of family 2C were descended had the sacral attachment shown in 5 and was therefore a type IV individual. Her mate was of the transition type shown in 2, figure 3, having twenty-six presacral vertebrae and with the coxal bone attached primarily to the twenty-seventh vertebra but in a slight degree also to the twenty-eighth vertebra. All the x-rayed descendants of this pair showed a tendency toward sacral shift backward at least equal to that of the father, and in many cases as great as that of the mother. The apparent difference between individuals of types II and IV as originally tabulated in this family (table 3a) is actually due then to the relative completeness or incompleteness of expression of a shift in sacral position. Considered from the standpoint of presence or absence of homeosis the individuals classified as of types I and II are in reality of types III and IV which accounts for the much higher percentages of progeny having extra lumbar vertebrae as well as extra ribs in this family, as compared with the other families.

In family 2A the study of the variations was carried out largely by dissection and unfortunately many of the specimens were not saved. Such data as are available, however, indicate that the lumbo-sacral shift observed in family 2A is not greater in degree than is shown in 2 of figure 2. However, even this small difference in degree of expression is probably sufficient to account for the small proportion of type IV individuals found in family 2A.

It seems evident from the above data that an explanation of the variation in number of presacral vertebrae as well as that of ribs may not be dependent as much upon agencies affecting the presence or absence of these units alone as it is upon underlying developmental agencies whose influence results in a graded series of expression in a general region. What these agencies are it is impossible at present to state, but the variation is such that it cannot be due to a single gene since neither the normal nor the abnormal condition can be said to breed true.

Family 3

Fewer observations than on either of the other families have been made upon still another race of large sized rabbits. It resembles family 2C in having among its progeny a high incidence of individuals having both extra ribs and an extra lumbar vertebra (type IV). It differs, however, in that the per cent of extra ribbed individuals is somewhat less, whereas the per cent of extra presacral vertebrae both in comparable matings and in the total progeny is much higher. A relatively high incidence of type III individuals is especially peculiar to this family, a type which, as shown in table 4, is very unstable, its progeny reverting principally to type IV, in a few cases to types I and II, but rarely being of type III.

TABLE 4

Classification of the young produced by matings in family 3

PARENTS	OFFSPRING					PER CENT HAVING EXTRA RIBS	PER CENT HAVING AN EXTRA LUMBAR VERTEBRA	PER CENT HAVING BOTH	DEGREE OF EXPRES- SION OF EXTRA RIBS
	I	II	III	IV	Total				
I × I	11	2	0	1	14				
I × IV	6	5	4	20	35	71.4	68.5	57.1	68.4
III × III	2	3	1	19	25	88.0	80.0	76.0	70.5
III × IV	0	0	0	18	18				
IV × IV	8	18	9	61	96	82.3	72.9	63.5	74.25
Totals	27	28	14	108	177	77.7	70.3	61.7	71.05

These data suggest the presence of agencies affecting the axial skeleton still farther posterior than those affecting either of the previously discussed families. This is especially evident when one compares the per cent of individuals having an extra lumbar vertebra in this family (70.3) with each of the other families none of which exceeds a total average of 51.0%, and two of which are much lower.

While there appears to be an increase in the per cent having extra ribs for each mating in this family, which corresponds to an increase in the per cent having an extra presacral vertebra, it is to be noted that the per cent of extra ribbed individuals is not in proportion but is actually lower than that of

family 2C. At the same time the expressivity of the extra ribs is—following the terminology of Timofeeff-Ressovsky ('34)—appreciably lower. Thus the causal agencies influencing homeotic variation in this family appear to exert a greater influence in the lumbo-sacral region than in the thoraco-lumbar. Whether this is due to the influence being centered at a point more posterior cannot be determined at present. If such were the case the magnitude of the influence must be proportionately greater since the per cent of extra ribs is not materially reduced.

Family 4

The most recent family to be studied is entirely unrelated to any of the others. It originated from an outcross of chin-chilla stock with the small-sized race of Castle and was bred father to daughter for several generations before this study began. Ninety-six individuals, including the original parents have possessed a normal thoraco-lumbar region. Two individuals were classed definitely as type III. One individual, a new type not previously encountered, with twelve ribs and six lumbar vertebrae, is to be interpreted perhaps as a step in the direction of vertebral reduction.

Although this family is 100% normal in the thoraco-lumbar region and the data show but 3% of abnormality as typed in the sacral region, close scrutiny reveals that in this family 88% of the individuals show more or less of a tendency for the fulcralis to involve not only the twenty-seventh vertebra, but also the twenty-eighth. Thus the family must be regarded as having a genetic constitution approaching closely the threshold for the production of twenty-seven presacral vertebrae.

RELATION TO SEX

There is reason to believe that one of the factors influencing the expression of extra ribs and presacral vertebrae is associated with sex. Many of the data presented in this paper have been obtained by dissection of newborn animals in which

sex is not readily established. However, as the study progressed it was found that descent or failure of descent of the gonad and the type of attachment could readily be used as a sex indicator. It has thus been possible to show a large proportion of the data so classified (table 5). Of a total of 2055 individuals, 1305 individuals have been examined outside of the above families. In general, there appears to be a slight tendency for males to exceed females in type I and for females to be in excess in type IV whereas in the other two groups the sexes are about equal. When grouped in such a way as to separate the four families described above (2B, 2C, 3 and 4), it appears that females are more likely to manifest either extra ribs or presacral vertebrae than males, but only when the manifestation of extra ribs is barely at the threshold of expression. Thus, in family 2B where extra ribs are relatively infrequent and poorly expressed, there is a noticeable but not statistically significant tendency for them to come to expression more frequently in the female. In family 2C, on the other hand, the threshold of expression is so low that male and female manifest the variation about equally. When ribs are present they are fully developed. In family 3 both ribs and extra presacral vertebrae are poorly developed and again the female expresses the variation more frequently than does the male.

It is interesting to note that the sex ratio of the total population conforms very closely to 104 males per 100 females described by Crew. In the general group apart from families 2B, 2C, 3 and 4, the number of males is slightly in excess. Whereas in these four families and particularly family 2B there tends to be a preponderance of females.

DISCUSSION

There is evidently a tendency in the domestic rabbit to increase the number of presacral vertebrae from twenty-six, which is the most common type, to twenty-seven. The degree and percentage of realization of this tendency can be increased by selection, which shows that it has a genetic basis. It finds

expression most frequently in the formation of an imperfect pair of ribs on the first lumbar vertebra. Simultaneously the sacrum may begin to shift its point of attachment backward to include a part of the twenty-eighth vertebra in addition to the entire twenty-seventh. Where this tendency finds fuller expression, the extra (thirteenth) pair of ribs is found more completely developed and the sacral shift toward the twenty-eighth vertebra is increased. When the tendency reaches its fullest expression both the thirteenth rib is well developed and the twenty-seventh vertebra is added to the lumbar region,

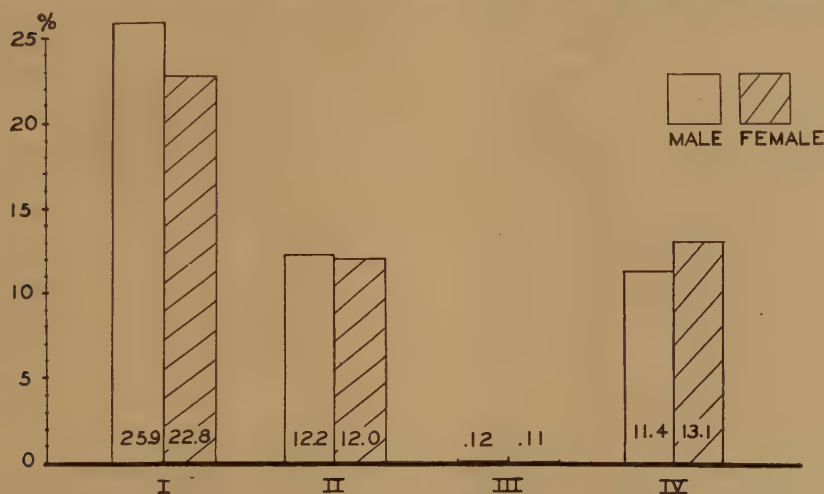


Fig. 3 Sex distribution of the four vertebral types in a total of 2055 individuals of all families.

the sacral attachment being then on the twenty-eighth. In some cases the attempt at sacralization of the twenty-eighth vertebra may occur without the development of extra ribs but the complete adjustment of the sacrum to the twenty-eighth vertebra alone is rarely attained without the addition of extra ribs. Since selection for either effect has thus far automatically served to increase the other the two processes must be correlated, although there is some reason to think that the development of extra ribs alone may be favored by a slightly different genetic background than that which favors the more posterior sacral attachment.

Neither the normal nor any of the abnormal states is entirely true-breeding even when selected for several generations, though each approaches such a condition.

It is an open question whether these developmental tendencies are governed by special genes or whether they are incidental consequences of such agencies as govern general body size. Since the female, which in the rabbit attains the greater size (Castle, '31) tends to possess the additional ribs and vertebrae the latter hypothesis is not without support. Such factors can hardly be the sole contributing influence, however, since a preliminary study of weight in these animals shows the maximum rib and vertebral development in family 2C, which is a medium-sized family; and that while one normal ribbed family (family 4) is small, one of the medium weight families (family 2B) likewise produces very infrequent abnormal progeny. In fact the mean weight of 2B slightly exceeds that of 2C.

Whereas in certain families, such as 2A, ratios approximating typical mendelian monohybrid F_2 and backcross proportions are obtained for one of the variations (twelve versus thirteen ribs), such an interpretation does not withstand the critical test of inbreeding the extracted recessives.

Although a manifestation of extra ribs and presacral vertebrae is more often found in females than in males, numerous examples could be cited to show that these variations may be transmitted by the male quite as well as by the female. As is shown in table 6, female 789 has been mated to her father (48D3), brother (3098) and son (4207), all four animals of which belong to family 2B and are normal in their rib expression. She has also produced a number of young in an outcross to ♂ (3880). Each of these matings is so significantly different as unmistakably to indicate inherent differences transmitted by the male concerned.

Whether this variation in the rabbit can be explained on a multifactorial basis such as that hypothesized by Promptoff to explain a similar variation in poultry seems doubtful at present in view of the extent of the variation and the diffi-

culty which has been encountered in securing homozygous stocks. The nature of the segregations observed in family 2A early led to the prediction of a dominant gene responsible for the rib variation. Somewhat later the type of variation encountered in family 2B, which it must be remembered, traces to a common ancestry with that of 2A, necessitated the assumption of a second genetic factor, dominant in nature, whose action serves to suppress the expression of the extra ribbed condition. But this addition to the gene complex, which makes it possible in two generations to reverse completely the

TABLE 6

Classification of young produced by ♀ 789 (type I) mated with four different males, which shows that the variation is transmitted by the male as well as by the female

MALE	RELATION	OFFSPRING					PER CENT HAVING EXTRA LUMBAR RIBS	PER CENT HAVING EXTRA LUMBAR VERTEBRA	DEGREE OF EXPRES- SION
		I	II	III	IV	Total			
48D3, type I	Father	38	12	..	..	50	24.0	0.0	50
3098, type I	Brother	17	1	..	..	18	5.0	0.0	50
4207, type I	Son	21	9	..	1	31	32.2	3.2	47.3
3880, type IV	Unrelated	4	5	..	6	15	73.3	40.0	69.2

apparent dominance of the variation, is still inadequate to explain all the variation without resort to additional modifiers, proof of which is at present incomplete.

The influence of environmental factors upon size and growth rates is well known. Kingsbury ('24) has pointed out that marked growth is attended by postponed or retarded differentiation and Hubbs ('26) called attention to the fact that retarded development in fishes is conducive to the production of extra somites, whereas accelerated development acts in a contrary manner. While it is generally assumed that intra-uterine environment in mammals is relatively constant the studies of Wright on polydactylous and otocephalous guinea pigs, and Reed on hairlip in mice, show that genetic and non-genetic influences are co-existent causal agencies in determining the expression of such characters.

It has been shown by Butcher ('29) that in the rat the somites from which the vertebrae arise are differentiated in numerical head to tail sequence, each succeeding one structurally more advanced and with differences in size, rate of formation and orientation. Any factor, genetic or otherwise, which may alter either the rate of growth or differentiation over a shorter or longer period may easily modify the expression of the vertebral type by increasing or decreasing the number of individual vertebrae of a given type. It may be of some significance that the formation of somites in the thoraco-lumbar and lumbar sacral regions approximate the time of implantation in the rabbit.

In more extreme axial variation such as rumplessness in fowl and the tailless condition in the rat and mouse, genetic factors have been well demonstrated and in the fowl (Danforth, '32) and the herring (Rounsefell and Dahlgren, '35) the effects of altered temperatures have already been noted. In the mouse and rabbit it has been demonstrated that the growth in specific regions such as the tail and ear is influenced by identifiable and in one case a well-known gene (Castle et al., '36 a, b).

Because of the close but inexact conformity to a mendelian interpretation and its apparent susceptibility to such minor environmental influences as are supposed to exist in the uterus in mammals, this variation should provide excellent material for further analysis of both general and specific genetic factors in growth and differentiation. It should also be of importance in determining the extent to which the uterine environment may influence the phenotypic expression of growth and differentiation of morphological characters.

CONCLUSIONS

1. Developmental tendencies operating in the thoraco-lumbar and lumbo-sacral regions of the axial skeleton of the rabbit which affect the presence and manifestation of either an additional pair of ribs, an extra presacral vertebra or both, are due to factors which are primarily genetic.

2. Neither a simple nor a multifactorial mendelian interpretation has been found which at present adequately accounts for all of the existing variation.

3. While additional skeletal units occur more often in females than in males they may be transmitted by the male quite as well as by the female.

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THE RELATION OF THE GOLGI MATERIAL TO THE SECRETORY PROCESS IN THE BASOPHILIC CELLS OF THE ANTERIOR HYPOPHYSIS ¹

ARTHUR J. GATZ

University of Minnesota and Carleton College

TWO PLATES (EIGHT FIGURES)

The possibility that the Golgi material might have a relation to the secretory processes of the cell has been postulated since the study of this structure began. Negri ('00) differentiated the Golgi material in the pancreatic cell from the intracellular secreting capillaries. Various interpretations have been given his work, some holding that he thought the Golgi material had a relation to secretion, others maintaining that he had made no such statement. Fuchs ('02) described a 'Fadenknauel' in the cells of the epididymis which he correlated with the secretory process. Prenant ('05) described a substance, the 'amas vermiculaire,' which had a direct contact with the secreted substance. Whether the above structures were the Golgi material may perhaps never be proved. However, von Bergen ('04) in work on cells of the prostate gland in the dog and the pancreas, sub-maxillary gland, and fundic stomach of the cat; Kolster ('13) in work on cells of the stomach, Brunner's glands, pancreas, and the thyroid of rabbits and dogs; and Cajal ('14) in work on cells of the submaxillary gland of the rabbit and the pancreas of the rabbit and cat observed morphological changes in the Golgi material which could be correlated with the secretory cycle of the cells.

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The development of the method of osmic acid impregnation has made more recent development of the subject possible. Not only is the Golgi material demonstrable, but the method permits an almost perfect demonstration of the secretory products. It was from preparations made in this manner that Bowen ('23 and '24) and Nassonov ('23) achieved their initial contributions. They used the pancreatic cells and the goblet cells of amphibians for their initial studies.

As a result of his work, Bowen concluded that many gland cells run through a regular secretory cycle. The cell is devoid of secretion granules in the beginning. Somewhat later the secretion granules form, mature, and are extruded during the cycle. The Golgi material hypertrophies and according to Bowen ('26) may be divided into three topographical types: 1) Serous, the Golgi material extends through the mass of secretory granules, having contact with all the granules. 2) Mucus, the Golgi material becomes basket-like, within which the secretory granules are enclosed. 3) Lipoidal, the Golgi material becomes scattered as discrete fragments throughout the cytoplasm.

The secretory granules arise in the area limited by the Golgi material. The secretory granules are considered to be differentiated by the Golgi material but no claim is made for the direct transformation of the latter structure into secretion granules, which has been done in the consideration of mitochondria and their relation to secretion.

Gatz ('33), Severinghaus ('33), and Guyer and Claus ('34) have made cytological studies of the anterior pituitary of the rat with special reference to the Golgi material. All three workers found that the Golgi material increased in size, that it (when present as a distinct body) was located between the nucleus and vacuoles, and that it apparently showed no polarization. Following gonadectomy, the basophilic cells gave rise to large vacuolated cells which the above workers assumed to represent the final secretory phase. Gatz proposed that there was a relationship between the Golgi material and secretion, citing the structures observed in the vacuolated basophiles.

Severinghaus maintained that neither the mitochondria nor the Golgi material could, with certainty, be related to either the elaboration or the discharge of the specific secretion. Guyer and Claus observed numerous globules in the strands of the Golgi material, which later appeared to pass out into the cytoplasm. They concluded that, "in the particular material with which we have worked, the vacuolar substance arises as a secretion within the Golgi framework or through its utilization as a center of transformation," and, "the vacuole is in some manner a product of the Golgi apparatus."

MATERIAL AND METHODS

The hypophyses of albino rats were used in this study. The glands were removed from animals which had undergone castration, vasectomy, ligation of the vas deferens, artificial cryptorchidism, and x-radiation of the testes, and also from normal and senile rats. Some of the glands were fixed in cobalt nitrate and neutral formalin without the subsequent silver impregnation (the method of Da Fano). The negative image of the Golgi material, which was exhibited by the above method, was of little value in this study. The other glands were impregnated with osmium tetroxide following the method of Mann-Kopsch (Weigl), and modified by Ludford. The details of the technique may be found in Guyer's 'Animal Micrology.'

It was necessary to fix the tissue immediately (1 to 2 minutes) after the death of the animal or the resultant impregnation with osmic acid was imperfect. The hypophyses were kept at a temperature between 33° and 34°C., during the period in which they were in osmic acid, under darkened and lighted conditions. The light factor seemingly had no effect upon the subsequent impregnation. The tissue was embedded in paraffin and serially sectioned at a thickness of 5 μ .

The sections which are prepared by the osmic acid method exhibit a cytoplasm which has a yellow-tan appearance. The nuclear membrane, which is stained brown, and the chromatin

granules, which are tinged brown also, outline the nucleus very clearly. The periphery of the Golgi material is much blackened and the lighter central portion of the body contains numerous osmiophilic threads and granules. It was often necessary to bleach the sections with a weak solution of hydrogen peroxide (2 drops of peroxide to 50 cc. of water) so that the minute details in the periphery of the Golgi material could be studied.

I wish to gratefully acknowledge my indebtedness to the director of this investigation, Dr. J. E. Wodsdalek, for his interest and suggestions during the progress of the work and the writing of the report and to the zoology departments of the University of Minnesota and Carleton College for the animals and materials which were supplied.

RESULTS

The majority of the animals used in this study either were gonadectomized or had the germinal epithelium present in a degenerate condition. However, numerous normal controls showed the same cytological picture though less frequently, since the pituitaries were not secreting as actively. Meyer, et al. ('30) by injections of oestrin into immature female rats; Reese and McQueen-Williams ('32) by injections of extracts from bull testes into male rats; Rogers ('33) by injections of theelin into female rats; and others have shown that gonadal hormones produced an inhibitory effect upon the hypophyses in their experimental animals. It has previously been shown that the Golgi material undergoes hyperthrophy following castration and artificial cryptorchidism in rats. Consequently, when the germinal epithelium is degenerate or has been removed, the inhibitory effect of the testes is absent. As a result, the chromophobe cells of the anterior lobe readily differentiate into basophilic cells. The basophiles, which in turn are not inhibited, readily enter an overactive secretory cycle.

The above results demonstrate the fact that the hypophyses of the experimental animals are ideal for the study of the secretory processes of the basophilic cells. In the present series of experiments, the Golgi material has undergone specific changes in size, shape and location in the basophilic cells. The Golgi material is usually present as a round or oval body, the periphery of which is very much blackened and frequently presents a net-like appearance. It is usually slightly separated from the periphery of the slightly eccentric nucleus (figs. 1 and 2), except in the case of the vacuolated cells. In the latter cells (figs. 6 to 8), the material is often smaller than in the basophilic cells of the normal animals and its position is irregular in the remaining rim of the cytosome. The material, often, is not exhibited as a distinct body, but appears as an accumulation of osmiophilic granules and thread-like particles. The particles, which are found scattered throughout the cytosome of the vacuolated cells, are especially numerous at the periphery of the vacuole.

The polarity of the Golgi material apparently is not of the importance which previous workers had attributed to it. In the present study, the Golgi material may be located on the side of the cytosome which is opposite the capillaries as frequently as it may be observed at the periphery of the cytoplasm which borders the capillaries. The distribution of the Golgi material in the vacuolated cells shows no indication of polarity.

The central portion of the Golgi material of almost all basophiles show some small granules of osmicated substance. These small granules were especially abundant in the animals which had been castrated or possessed degenerate germinal epithelium. These same cells also exhibit small secretion droplets, which are surrounded by a girdle of osmiophilic substance derived from the Golgi material, within the central portion of the 'body' (figs. 3, 4 and 5). The material within the droplets is of the same nature as that which is contained in the large and small vacuoles which are abundantly found in the vacuolated basophiles of the experimental animals. The

basophiles of the normal control animals showed similar osmiophilic girdles which never were, however, as numerous as in the experimental animals. These results, seemingly, are some of the initial steps of the secretory cycle in the basophilic cell of the anterior hypophysis.

The Golgi material presents another appearance almost as frequently as that described above which may also be considered to be a part of the secretory cycle. The osmicated periphery of the 'body' exhibits numerous small evaginations of varying sizes and within which small droplets of different sizes are to be seen (figs. 1, 2 and 3). Frequently a small vacuole forming within the cell is partially surrounded by these evaginations of the Golgi material in which secretion droplets are present. In many of the larger basophilic cells, small vacuoles which are forming have their periphery lined with a number of these osmiophilic girdles, which apparently have migrated either from the periphery or the central portion of the Golgi material. The small evaginations on the periphery of the 'body' are identical in structure to those which have been previously described within the central portion of the Golgi material.

In a similar manner, some of the larger vacuoles within the large vacuolated cells exhibited the same type of droplets, enclosed by osmiophilic girdles, lining the periphery of the vacuole (figs. 6 and 8). In these cells, the osmiophilic girdle and the contained secretion droplets were larger in size than those displayed within the Golgi network.

The actual secretion or the entrance of the droplet into the large vacuole was not observed. However, the result of the secretion into the vacuole was observed in the cytosome about the periphery of the vacuole. There are numerous granular, semi-circular, or thread-like particles of osmiophilic substance scattered in the cytosome bordering the large vacuole (figs. 6, 7 and 8). These evidently, have been produced by the collapse of the osmiophilic girdle accompanying the elimination of the secretion. In the larger vacuolated cells, the majority of the Golgi material has apparently been

utilized for secretory purposes since the 'body' usually is absent. The only remnants of the Golgi material which are present are the fragmented particles and the osmiophilic girdles which have already been described as located in the narrowed rim of the cytosome (fig. 6).

DISCUSSION

The modifications of the Golgi material which have been described were not as numerous in the glands of the normal rats as they were in the glands of the experimental animals. This fact is in accord with the knowledge of the present day. Numerous workers have shown that the injection of sex hormones inhibited the secretory processes of the basophilic cells. The writer ('36), as well as others, have shown that the loss of the germinal epithelium causes an increase in the number of the basophilic cells. The latter cells undergo an active secretory cycle which is shown by the hypertrophy of the Golgi material and the appearance of the numerous vacuolated cells.

It was during the above study that the writer observed the appearance of the osmiophilic girdles containing the secretory droplets. The function of these cellular components, which have been found intimately associated with the Golgi material and with the vacuoles of the basophilic cell, can be postulated in the following manner. The osmiophilic girdle, which is derived from the Golgi material, either serves an enzymatic function or acts as an intracellular center for chemical synthesis. The supposition that the Golgi material serves an enzymatic function during the secretory cycle of the cell affords an explanation for the elimination of the distinct 'body' in the vacuolated cells.

Enzymes or catalysts are known to function more efficiently as their composite surface is increased by fragmentation. The use of the same principle within the cell would result in the division of the Golgi material into smaller granules and girdles which have been shown to be present. It may be readily seen that the formation of rings of osmiophilic substance from the granules would afford the maximum area of

contact with the forming secretion droplet. The presence of the fragmented portions of the osmiophilic girdle causes one to conclude that the substance is a center for the synthesis of the secretion. It also confirms the fact that the Golgi material does not become transformed into a secretion droplet, a process in which the Golgi material would lose its identity.

SUMMARY

1. The results of the study show a definite interrelationship between the Golgi material and the 'secretory cycle' of the basophilic cell which may be outlined as follows:

a. There are osmiophilic granules within the central portion of the Golgi material.

b. Secretion droplets, of varying sizes, are surrounded by an osmiophilic girdle within the central portion of the Golgi material.

c. There are also droplets in the osmiophilic periphery of the Golgi material.

d. Secretion droplets are surrounded by an osmiophilic girdle at the periphery of the small forming vacuoles, as well as at the periphery of the large vacuoles in the vacuolated cells.

e. Osmiophilic granules and particles are located in the cytosome of the large vacuolated cells. These fragments are apparently the remnants of the osmiophilic girdle which had previously surrounded the secretion droplet.

2. The Golgi material apparently serves an enzymatic function or acts as an intracellular center for chemical synthesis.

3. The Golgi material of the basophilic cell does not become transformed into a secretion droplet. The osmiophilic fragments, which are derived from the osmiophilic girdles, are derived from the 'body.'

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PLATE 1

EXPLANATION OF FIGURES

1 Basophiles from anterior hypophysis of normal control rat. The Golgi material is marked by the blackened periphery of osmiophilic substance. Note the small droplets of secretion in the central portion of the one cell and the marked peripheral evaginations of the Golgi material in the lower cell. $\times 1800$.

1 a Camera lucida drawing of Golgi material in upper basophile of above figure.

2. Basophile from anterior hypophysis of rat 15 months after castration. Numerous small secretion droplets are present in the periphery of the Golgi material. $\times 1800$.

2 a Camera lucida drawing of Golgi material in the basophile of figure 2.

3 Basophile from anterior hypophysis of rat 20 days after x-radiation of testes. The peripheral portion of the Golgi material contains numerous secretion droplets. The photograph was taken slightly out of focus to show the large number of droplets sometimes present in a cell. $\times 1750$.

3 a Camera lucida drawing of Golgi material in the basophile of figure 3.

4 Basophile from anterior hypophysis of rat 30 days after x-radiation of testes. This cell shows the osmiophilic girdles in the central portion of the Golgi material as well as the evaginations in its periphery. $\times 1750$.

ABBREVIATIONS

OG, osmiophilic girdle

SD, secretory droplet

OGF, fragmented particles of osmiophilic girdle

V, vacuole

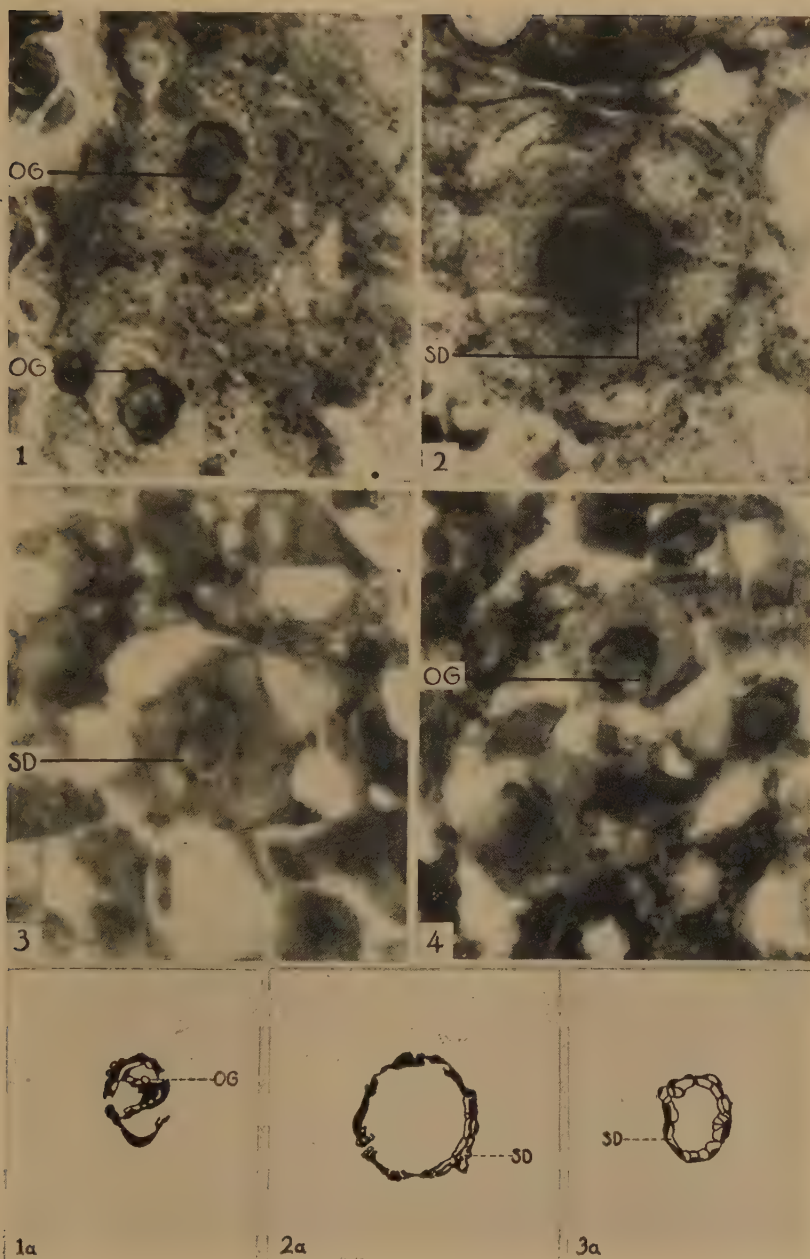


PLATE 2

EXPLANATION OF FIGURES

5 Basophile from anterior hypophysis of rat which had had testes x-rayed 2 months previously. The periphery of the Golgi material is not in focus. Observe the numerous secretion droplets which are surrounded by an osmiophilic girdle in the central portion of the Golgi material. $\times 1750$.

6 Vacuolated basophile from rat which had been castrated 2 months. The Golgi material is not present as a 'body.' Observe the osmiophilic girdles and the fragmented particles of osmiophilic substance lining the periphery of the vacuole. The nucleus is the small light oval body in the cell. $\times 1800$.

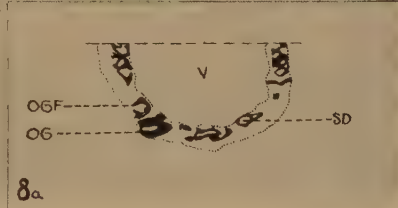
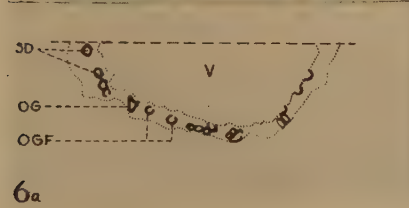
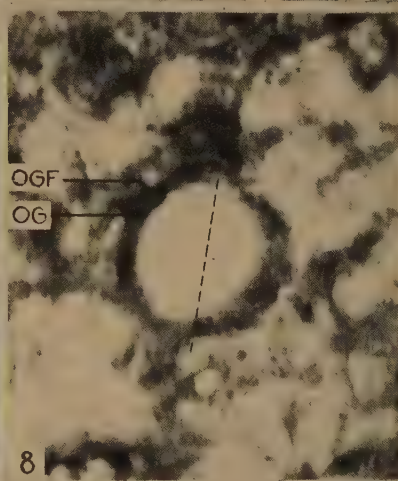
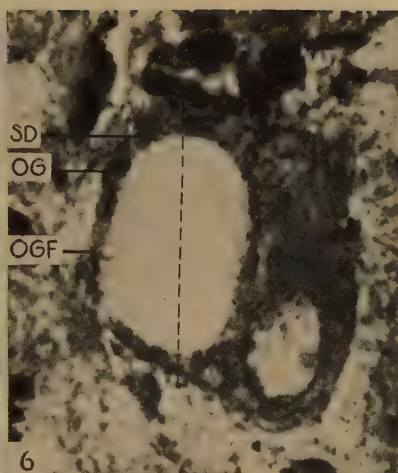
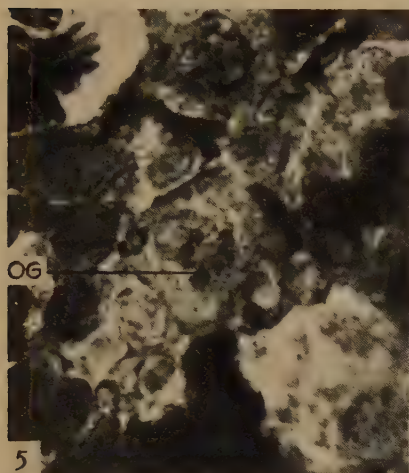
6a Camera lucida drawing of portion of figure 6, which is demarcated by vertical line.

7 Vacuolated basophile from rat which had been castrated 15 months. The description is the same as for the above figure. $\times 1800$.

8 Vacuolated basophile of rat 2 months after x-radiation of testes. There are numerous osmiophilic girdles in the cytoplasm around the large vacuole. $\times 1750$.

8a Camera lucida drawing of vacuolated cell in figure 8, which is demarcated by vertical line.

The drawings are a more or less diagrammatic representation of the osmiophilic girdles and the contained droplets found in the Golgi material.



AN EARLY STAGE OF HUMAN IMPLANTATION

JOHN STEPHENS LATTA AND J. PERRY TOLLMAN

Departments of Anatomy and Pathology, University of Nebraska College of Medicine, Omaha

ONE TEXT FIGURE AND THREE PLATES (FOURTEEN FIGURES)

Some time ago a specimen of a uterus surgically removed from a sexually active woman was sent to the pathology laboratory for examination. It was found later that the endometrium contained an ovum in an early stage of development. Careful examination of the implanted tissues revealed no traces of embryonic disc, amnion or yolk sac. Nevertheless histological study indicated that the tissues were very well preserved and several interesting features of implantation were well shown. In view of the fact that only relatively few ova in situ in such an early stage of development have been described, we have thought that an account of the features presented by our specimen would prove interesting, even though necessarily incomplete.

CLINICAL HISTORY

A married white woman, aged 40, entered the University Hospital complaining of back ache, frequency, urgency and irregular periods. Her past history showed three spontaneous miscarriages and marked menstrual irregularities over a period of 8 years. The periods had been generally at 28-day intervals, one to four periods occasionally being missed, until 11 months before the present specimen was obtained. Since that time they had occurred at 20-day intervals. A curettage 5 months before the present admission showed normal endometrium. The uterus measured $6 \times 5 \times 5$ cm. The endometrium averaged 4 mm. in thickness and exhibited no gross changes.

MICROSCOPIC PREPARATION

After gross examination of the endometrial surface and before any fixation of the tissue, a thin section of it extending to and including some of the muscularis was cut out with a safety razor blade, fixed in formalin, dehydrated in alcohol, cleared in chloroform, infiltrated and embedded in paraffin. Inspection of the cut edge of this block of tissue had shown a small cyst about 5 mm. in diameter just beneath the endometrial surface. A sample section was cut from this block and stained routinely at the hospital laboratory with hematoxylin and eosin. Examination of this section showed that this cystic structure was a chorionic vesicle in a very early stage of implantation and development. The remainder of the block of tissue was immediately presented to the department of anatomy together with blocks of tissue removed from the endometrium on either side of the region from which the original block was removed, both of which were found to contain portions of the chorionic vesicle. The original cuts in the fresh endometrial surface had, therefore, cut directly through the embryonic membrane. Serial sections were made of each of these blocks of tissue, some of the sections being $10\ \mu$ and others $15\ \mu$ in thickness. Most of the series was stained with Mayer's hematoxylin and eosin, a few slides being stained with Delafield's hematoxylin and azur II-eosin.

The sections cut from these three different blocks of mucosa were arranged as accurately as possible to make one nearly complete series through the implanted ovum. Where the series from one block passed over into the adjacent one some tissue was lost, and there were undoubtedly slight changes in the plane of sectioning of the three pieces. At the beginning and end of each block incomplete sections were cut, all of which were mounted as part of the series. These factors made the determination of measurements rather inexact, although we believe that the measurements given are very nearly correct.

GENERAL DESCRIPTION

Our first examination of the implanted ovum showed that it was completely covered by a layer of decidua 0.14 mm. thick after fixation. Unfortunately it was found that an artificial break had occurred, probably during manipulation of the fresh tissue, which extended through the covering decidua and the underlying chorion into the chorionic cavity. In addition and possibly as a result of this rupture of the vesicle there was found to be considerable distortion of the chorionic mesoderm, consisting principally of withdrawal of the mesodermal cores of the shorter villi and occasional breaks without displacement in the chorionic epithelium. In view of the necessary method of preparation and these evidences of distortion, we were not surprised, though greatly disappointed, that we have been unable to discover any traces of embryonic disc, amnion or yolk sac.

Regardless of the above-mentioned evidences of distortion, the chorionic vesicle appears to have been relatively undisturbed, having retained a practically spherical shape, and the chorionic-mucosal relationships have remained intact. The developmental state of the chorion and the size of its cavity, together with its position in and relations to the uterine mucosa, give ample evidence that this material presents a very early stage of implantation.

MEASUREMENTS AND RELATION TO OTHER DESCRIBED
EARLY IMPLANTATIONS

In order to more accurately determine the size of the implantation cavity and the limits of the embryonic trophoblast, several photographs were taken of sections through the center of the vesicle. Measurements were made from the photograph in which the vesicle was of greatest diameter. In this section there was some displacement of the edges of ruptured chorion previously mentioned and overlying decidua capsularis (fig. 1). In determining the diameter of the vesicle in a plane perpendicular to the surface, measurements were taken from both of these broken edges. The average of these

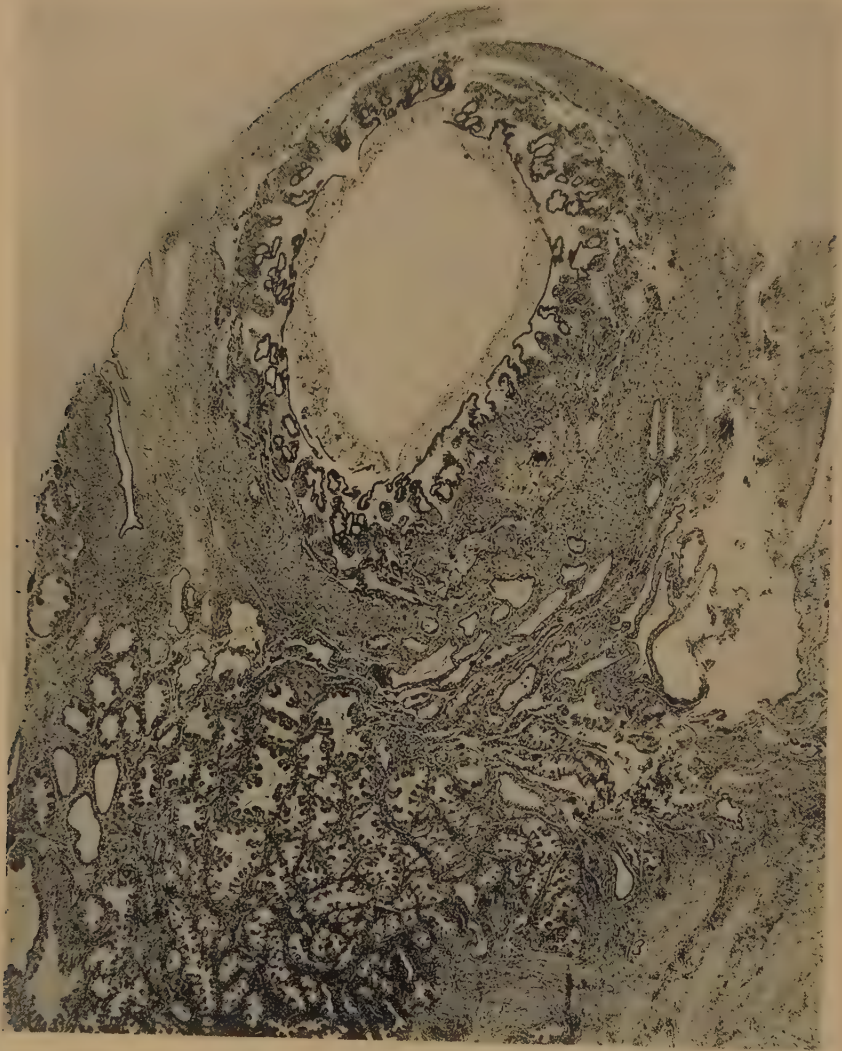


Fig. 1 A section near the center of the implanted ovum. The enlargement of the uterine glands and the numerous papillary folds are clearly shown. Note the comparative absence of glands in the more compact tissue immediately surrounding the implantation cavity and also compactness of the glandular tissue in the region immediately underneath the ovum. $\times 18$.

two measurements give a vertical diameter of 4.25 mm. The greatest diameter in a plane parallel to the endometrial surface as measured from the same photograph was 4.54 mm. Estimation of the diameter of the vesicle in a line perpendicular to the plane of section was inexact because of the incomplete sections at adjacent edges of the three blocks of tissue. Our computation of this measurement from the sections gave a diameter of 5.5 mm. The external dimensions of the chorionic vesicle, including the trophoblast, and likewise the dimensions of the implantation cavity were approximately $4.25 \times 4.54 \times 5.5$ mm. These values are quite similar (slightly less) than those computed by Stieve ('26) for 'Ei Hugo' and less than those given for the Mateer (Streeter, '20) and the von Herff (Von Spee, 1896) embryos, somewhat larger than for the embryo described by Kindred ('33) and very nearly the same as those given for the Greenhill specimen ('27).

True villi were present, extending from all sides of the chorionic surface. These were considerably longer and slightly more complex in the basal and marginal zones than in the region of the covering decidua capsularis. In no instance, however, was there any great degree of complexity, there being few indications of branching and the largest villus measured being slightly less than a millimeter in length. Compared with other ova of similar stages of development, the villi of this specimen do not appear to be as long and complex as those of the Mateer, Carnegie no. 763 (Streeter, '20), or the Goodwin embryo of Kindred ('33); but somewhat more complex than represented for the Greenhill specimen. In respect to chorionic development our case apparently most closely resembles the 13½-day-old embryo described by Stieve ('26), although his illustrations of this feature are inadequate for exact comparison.

Measurement of the diameter of the chorionic cavity was made only from a photograph of the largest section in planes parallel and perpendicular to the mucosal surface, the dimensions not including the mesoderm being $2.6 \times 2.6 \times 3.7$ mm.

From the incomplete data available in the specimen here presented, we have concluded that it should be considered as a younger example of ova of group II, stage 4 of Streeter's classification ('20) and class C 1 of Grosser's classification ('24). Therefore the embryo associated with these adnexa, which was so unfortunately lost must have been in a pre-somite stage, probably exhibiting a primitive streak in the embryonic disc.

UTERINE MUCOSA

The mucous membrane in general was somewhat thickened and presented an appearance quite similar to the mucosa in the premenstrual phase, as observed by several others coincidental to early stages of implantation (e.g., Linzenmeier, '14; von Möllendorff, '21; Stieve, '26; Streeter, '26). The connective tissue stroma was very edematous and markedly infiltrated with polymorphonuclear leucocytes. The glands were very large and tortuous, with numerous papillary folds extending into their lumina (fig. 1). The glandular epithelium was columnar and had evidently been actively secreting, as shown by secretory globules extending into the lumen from many cells and the presence of secretory products within the glandular lumina (as described by Linzenmeier, '14; Falkiner, '32). The stroma appeared as columns between the vertically placed glands, and in the columns or septa were found many sections of small, thick-walled arteries. The stroma was less edematous and the glands more closely packed, with increased tortuosity and folding immediately underneath the basal zone of implantation (fig. 1).

Approaching the tissues immediately surrounding the chorionic vesicle the glands were found to have changed their direction so as to go around the area of implantation (Teacher, '25; Greenhill, '27). The ovum with its covering of decidua was shown by the sectioned material to have extended into the uterine lumen behind the general surface. This covering decidua capsularis, as stated earlier, was complete and of a thickness of 0.14 mm. There was no fibrin plug,

discontinuity of the covering epithelium (as in the ovum described by Tennant and Ramsay, '34) (except where artificially ruptured in handling), or operculum of trophoblast cells or other evidence of the locus of the portal of entry as were found by Falkiner ('32), Stieve ('26), Greenhill ('27) and Kindred ('33) in implantations of about the same age and described in detail by Teacher ('25) in younger stages. We did note in this case, as observed by von Möllendorff ('25) that the surface epithelium, as well as the underlying connective tissue cells over the capsularis, was much flattened.

In the zone immediately outside the trophoblast (the 'Umlagerung' zone of some German investigators) the connective tissue seemed more compact than elsewhere. Very few glands were encountered, and in those found there was little tortuosity and no papillary folds (fig. 1). A lack of uniformity in the height of the epithelial cells was encountered, although generally it was lower than in glands encountered elsewhere. In the glands nearest to the trophoblast, the epithelial lining was often incomplete and the cells somewhat piled up. From the shrunken appearance of the nuclei and the rather indefinite cytoplasmic staining, it is thought that this heaping up of cells was a part of a degenerative process. Occasionally cell strands extend through such breaks into the glandular lumen, the strands being traceable directly to the embryonic trophoblastic syncytium (fig. 3).

Detailed examination of the connective tissue in this surrounding zone revealed that the stroma here was quite edematous. There was possibly some increase in size of the connective tissue cells (Linzenmeier, '14), but we could find no evidences of definitive decidual cells and stages leading to them such as von Möllendorff ('25) described in embryo 'WO' only in the marginal zone. The infiltration with polymorphonuclear leucocytes previously noted was more marked here than elsewhere, but we also found numerous pycnotic nuclei and other signs of degeneration of connective tissue cells (fig. 2). There was, however, no necrotic zone as found in earlier stages by Bryce and Teacher (Teacher, '25), von

Möllendorff ('21) and others. Strands of syncytial tissue extended for some distance into the maternal tissue from the general trophoblast boundary. Many of these consisted of large multinucleated masses with deeply staining cytoplasm characteristic of the embryonic syncytium bordering the intervillous space. Many of the nuclei of these trophoblastic elements were very pycnotic, indicating degeneration. These invasive elements gave little demonstrable evidence of ability to digest the mucosa proper (fig. 2). Most investigators of early stages of human implantation have described a marked vascularity of the surrounding maternal tissue. The present example is no exception in that numerous large endothelial lined sinuses were encountered in this zone in very close relationship to the outer limits of the trophoblast. There were numerous evidences in the sectioned material of the disruption of the endothelial lining of some of the spaces by extensions from the syncytial trophoblast, in a few cases the syncytium forming one wall of the sinus for a short distance. In such regions there was usually a direct communication between the vascular lumina and the intervillous space (fig. 4).

CHORION AND TROPHOBLAST

The chorionic vesicle which was enclosed by this specially differentiated zone of the endometrium had been ruptured in preparation, as stated earlier, and the lumen contained nothing except a few maternal blood corpuscles and two fragments of villi. The chorionic wall was made up of a thin layer of mesoderm covered by a double layer of ectodermal cells (trophoblast). The chorionic villi projecting from the wall were variable in length and complexity, the greatest development being in the basal and marginal zones. In no instance was there found any great degree of complexity in branching. The mesoderm had been withdrawn either partially or completely from the villous cores, causing the mesoderm lining the chorionic vesicle to appear thicker than it actually was and those villi themselves to appear as hollow ectodermal tubes, continuations of the basal trophoblast. From the distal

ends of these true villi extended massive columns of trophoblastic cells, which, as they approached the surrounding mucosa, spread out and fused with distal ends of adjoining cell columns to form an incomplete layer of trophoblast cells ('trophoblast shell') (fig. 7) in juxtaposition to the surrounding maternal tissues. Usually the peripheral trophoblast lay directly upon the decidual tissue and was easily distinguishable from it by differences in staining reaction. The two opposing tissues were intermingled in certain regions where long streamers from the syncytial trophoblast extended into the decidua, including maternal blood vessels and glands (as described earlier). There were frequent interruptions in this outer layer of trophoblast, most or all of which formed communicating channels between maternal blood sinuses and the intervillous spaces.

Upon detailed examination of the two-layered trophoblast which clothed the basal chorion and the sides of the mesodermal villi, it was seen that the epithelial nuclei lying next to the embryonic mesoderm were comparatively small and regularly arranged, with chromatin knots evenly dispersed. In the basal chorion the cytoplasm of these layers was somewhat granular, but the cell membranes were usually heavy and distinct (fig. 5). Along the sides of the villi the cytoplasm of the cells composing this layer, the cytotrophoblast, was often entirely clear, with cell membranes correspondingly more prominent (fig. 6) and numerous mitotic figures were found in this layer, particularly along the sides of the villi.

Covering this layer and bordering the intervillous space, there was a syncytial layer of granular, deeply stained cytoplasm in which no cell membranes were demonstrable and nuclei were irregularly interspersed. These nuclei were usually very large and vesicular with prominent nucleoli and heavy nuclear membranes (fig. 5). Along the villi the nuclei were sometimes quite regularly arranged, smaller and not so vesicular, resembling quite closely those of the internal cytotrophoblast (fig. 6). Occasional cells with very clear cytoplasm, evidently originating from the cytotrophoblast, were found in the midst of the syncytial layer (plasmotrophoblast).

From the terminal ends of the true villi thick columns of cells extended toward the end of the implantation cavity. Near the villus these cells were closely packed with no intercellular space. They were in direct continuity with the cytotrophoblast and presented the same appearances of clear cytoplasm, prominent cell membranes and nuclei with evenly distributed chromatin characteristic of the inner epithelial layer of the villus (fig. 8). Mitotic figures were occasionally encountered in this region. Proceeding further from the ends of the villi, the cells comprising these columns became more spread out; so that spaces were apparent between some of them. Other cells in this part of the column became separated from the general mass so as to lie in spaces; such cells having very indefinite, degenerate cytoplasm and either very faded or shrunken and pycnotic degenerate nuclei, giving the impression that the spaces arise in the cell columns through degeneration of some of the cells. The cytoplasm of the remaining cells was quite stretched out and more deeply stained, cell membranes were rarely demonstrable and the nuclei much larger and more vesicular than in the cells nearer the villi. In many of these larger nuclei prominent nucleoli were observed. It was apparent that the cellular tissue here was intermediate between typical cytotrophoblast and plasma trophoblast. Some of the space found between the cells here had established connection with the intervillous spaces as shown by the presence of maternal blood cells in the former (fig. 9).

Covering most of the cell cords and separating them from the definitive intervillous space, there was a layer of typical syncytial trophoblast, a continuation of that covering the cytotrophoblast of the true villi. This layer was somewhat incomplete so that at some places the typical or transforming cytotrophoblast was directly exposed to maternal blood in the spaces in the trophoblast or the definitive intervillous spaces (fig. 7). From the covering syncytial layer of the villi and cell cords there were frequent extensions of syncytial masses into the intervillous space. Approaching the surrounding decidua

the amount of syncytial trophoblast increased markedly; so that we found no place in which the cytotrophoblast was in contact with the decidual tissue, an incomplete layer of syncytium intervening. These large masses of syncytium had sometimes apparently lost contact with the layer covering the villi and cell cords and lay as isolated masses in the implantation cavity. The nuclei in such masses presented great variety in size and distribution. Some were extremely large, vesicular and widely separated from each other, others quite small and very closely clumped together (fig. 10). In some of these syncytial masses near the basal side, spaces or vacuoles of great variety as to size and number were found. Nuclei of such syncytial masses were usually quite shrunken and pycnotic. Irregular projections from the surface of some masses were found in which the typical deeply stained cytoplasm had faded, groups of deeply stained granules being present (fig. 11). Some such degenerating masses containing no nuclei were apparently lying free in the intervillous space. There were also found large syncytial masses near or adjacent to the maternal tissue in which both cytoplasm and nuclei had almost completely faded out. The degenerative forms of syncytium surely indicate a regressive condition and should be designated as 'symplasmata' as according to Linzenmeier ('14), Grosser ('12) et al. We are undoubtedly in this case dealing with the degeneration of excessive syncytium of the second generation, the resorption syncytium (Grosser, '22; Fetzner and Florian, '30). From our study in this specimen, in which the majority of the trophoblast exposed to maternal blood was syncytial in character, that transformation of cytotrophoblast into syncytium was dependent upon contact with maternal blood or decidual tissue, as predicated by Peters in 1899 (see Grosser, '12). This transformation might further be considered as of the nature of a degenerative process which, in the exuberant masses of syncytium, is carried on to complete disintegration.

It is, of course, the layer of syncytium immediately covering the mucosa that gives origin to the long radiating syncytial

strands extending for considerable distances into the maternal connective tissue (fig. 2), disrupting glandular epithelium (fig. 3), and invading and partially lining maternal blood vessels (fig. 4) as described previously.

The mutual antagonism mentioned by von Möllendorff ('21) in his study of the 'Sch' embryo is further shown by the evidences of degeneration in the syncytial strands penetrating the maternal connective tissue and by the pycnotic nuclei so frequently encountered in the cells of this connective tissue, as well as the disrupted glands and blood vessels. We found almost no fibrin in the surrounding zone, such as described and illustrated in embryos described by Greenhill ('27), Stieve ('26), Falkiner ('32) and many other investigators in younger embryos.

The absence of a necrotic zone in the uterine stroma, its penetration by syncytial tissues, together with the absence of any space around the penetrating syncytial elements indicate that the ovum is entering upon a phase during which it will become anchored to the surrounding decidua.

CHORIONIC MESODERM

Adequate study of this tissue was impossible because of the great distortions encountered which had occurred in preparation. Next to the chorionic cavity was a thin layer composed of spindle-shaped cells, arranged parallel to the chorionic surface and lying in a practically homogeneous matrix. The few evidences of fibers seen were considered to be finely drawn out cell processes. This layer was definitely set off from the chorionic cavity which contained no magma, but no complete surface layer of mesodermal cells could be demonstrated. The spindle-shaped cells possessed elliptical nuclei with finely divided and evenly distributed chromatin. Some small groups of three or four partially rounded up cells were encountered, containing spheroidal, vesicular nuclei with prominent nucleoli. Individual spherical cells were found lying free in the meshwork, containing large nuclei with chromatin collected around the nuclear membrane and definite

central nucleoli, but otherwise clear. The cytoplasm of such cells was stained rather deeply with basic stain (fig. 12). Cells with quite long processes were seen containing similar nuclei. These were most numerous in the mesoderm adjacent to the cytotrophoblast.

The mesodermal cores had been to a great extent either partially or completely withdrawn (figs. 1, 7 and 13). Masses of mesoderm lying next to the epithelium in which the fusiform cells lay perpendicular to the surface undoubtedly represented mesoderm originally within the villi. When the mesoderm was only partially withdrawn from a villus it could be seen to have separated cleanly from the cytotrophoblast and in villi in which the mesoderm was intact there was a distinct line of separation between it and the overlying epithelium (fig. 13). We failed to find any evidence in this case to indicate that either angioblastic or mesodermal cells were arising from the cytotrophoblast as shown by Hertig ('35) in embryos of a similar stage and in macaque embryos. Cells comprising the villous mesoderm were of the same nature as those encountered in the basal mesoderm. Cells in various stages of rounding up to become free, possessed vesicular nuclei and nucleoli were quite numerous (fig. 14). Groups of three or four free cells with basic staining cytoplasm and large vesicular nuclei were sometimes found here also (fig. 15). Throughout the mesoderm, particularly in the villi, there were some strands of larger fusiform or stellate shaped cells with large vesicular nuclei with nucleoli and rather deeply staining cytoplasm which might be interpreted as the endothelium of beginning vasculogenesis (figs. 13 and 15) (see Stieve, '26; Hertig, '35). Considering the distortions encountered in the prepared tissues, we do not feel justified in drawing conclusions as to the nature of the single or grouped free spherical cells, although they have many features characteristic of primitive blood cells. Intermediate cell stages in the chorionic mesoderm indicate, surely, a mesodermal origin for both these free spherical cells and the endothelial (?) strands.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

2 A portion of the maternal mucosa lying immediately outside the implantation cavity, showing invasion by deeply staining strands of syncytial trophoblast, the nuclei of which are mostly quite pyknotic. One large trophoblastic mass is shown which contains several typical nuclei. There are no indications of any histolytic activity on the part of this trophoblast. Note evidences of degeneration of connective tissue cells and polymorphonuclear infiltration. $\times 600$.

3 The lumen of a gland being invaded by a strand of syncytial trophoblast. The disorganization and piling up of the epithelial nuclei is quite apparent. $\times 600$.

4 This shows the invasion and partial lining of a maternal blood sinus in the marginal decidua by syncytial trophoblast. Decidua above, trophoblast and implantation cavity below. $\times 78$.

5 Cellular detail of the cytotrophoblast and the syncytial trophoblast (x) in the basal chorionic epithelium. Note the distinct cell membranes of the cytotrophoblast. Compare the nuclear appearances in the two layers. $\times 1340$.

6 Junction of a villus with the basal chorion. In the covering of the villus many of the nuclei in the syncytial layer closely resemble those of the cytotrophoblast. The mesodermal core of the villus has been partially retracted. $\times 600$.

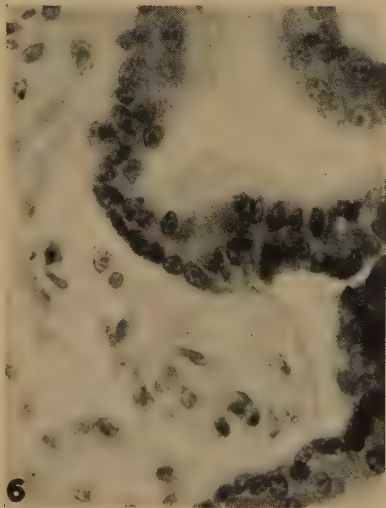
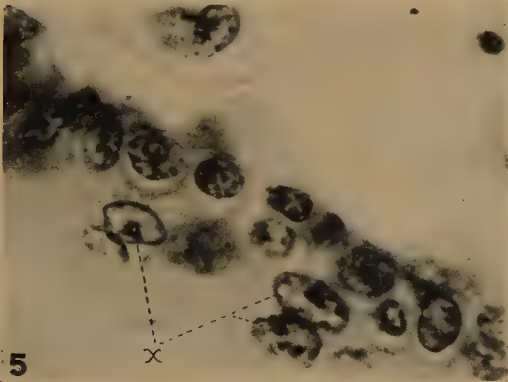
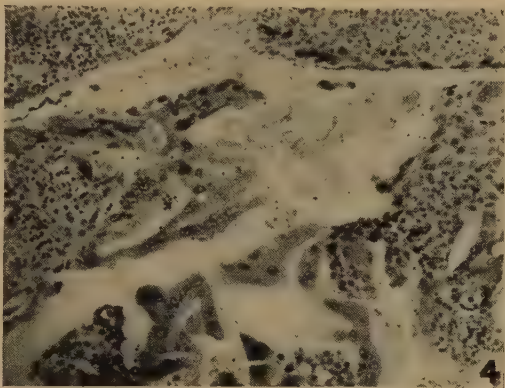
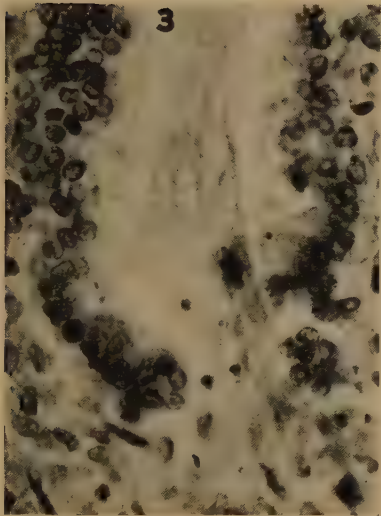
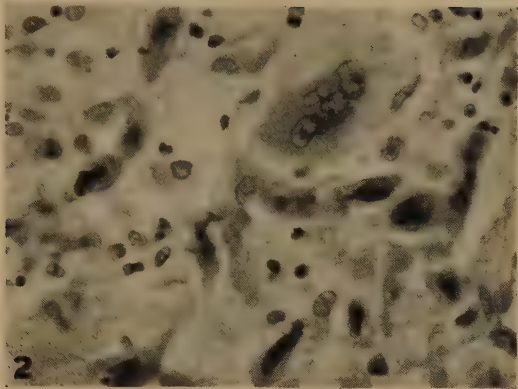


PLATE 2

EXPLANATION OF FIGURES

7 Cell columns continuing from the terminal ends of chorionic villi and uniting together peripherally. The mesodermal cores of the villi have been retracted in preparation. $\times 78$.

8 Tip of a chorionic villus and the proximal end of a cell column in continuity with it. The cells of the cytotrophoblast clothing the villus are identical with those comprising this portion of the cell column. Villus core (x). $\times 600$.

9 The character of the cells making up the column somewhat peripheral to region illustrated in figure 8. The cytological characteristics shown here are indicative of transition between cytotrophoblast and syncytium. The appearance of spaces due to the degeneration of certain cells is clearly indicated. $\times 600$.

10 Syncytial trophoblast from the periphery of the implantation cavity. Note the great variation in the concentration of nuclei in the protoplasmic mass. $\times 600$.

11 Syncytial trophoblastic masses projecting into the intervillous space. In the lower mass the nuclei are shrunken and pyknotic and large vacuoles are present. Projecting from the mass at the upper right is a non-nucleated fragment (x) containing numerous deeply staining granules. Much of the debris in the intervillous space appears as disintegrating syncytial tissue. $\times 600$.

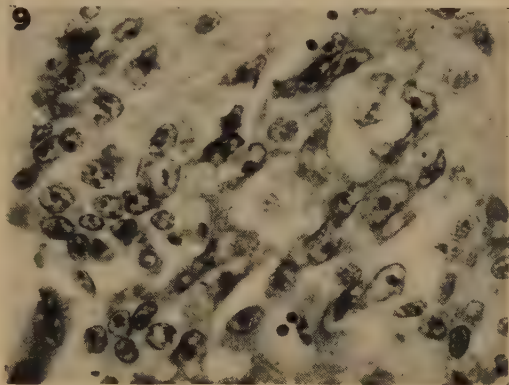
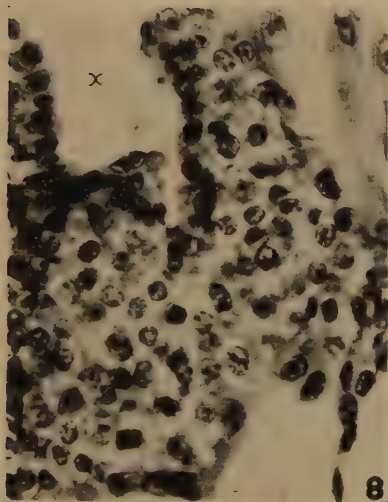
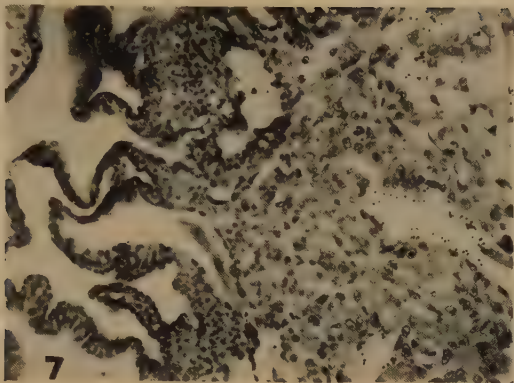


PLATE 3

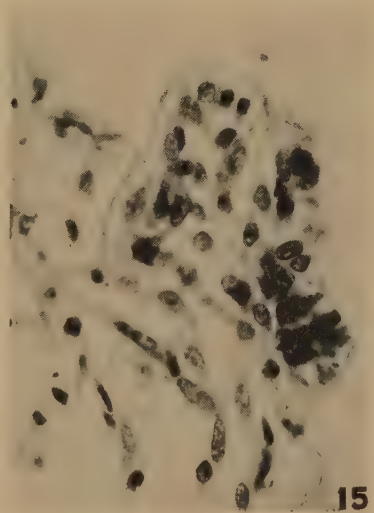
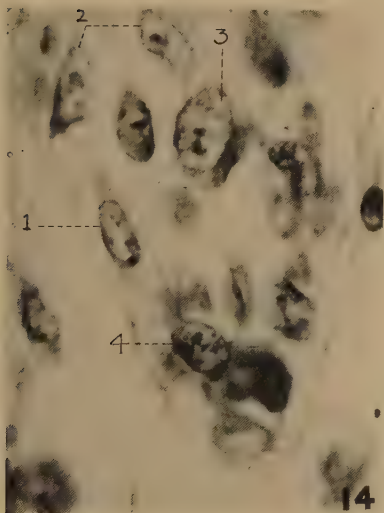
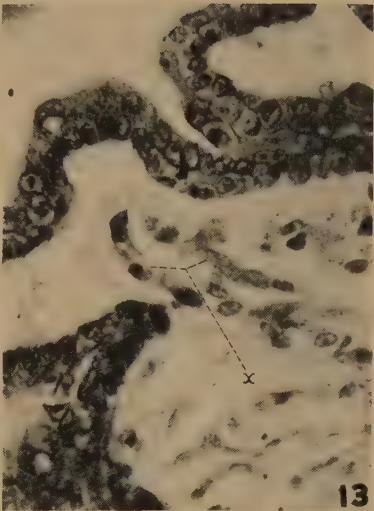
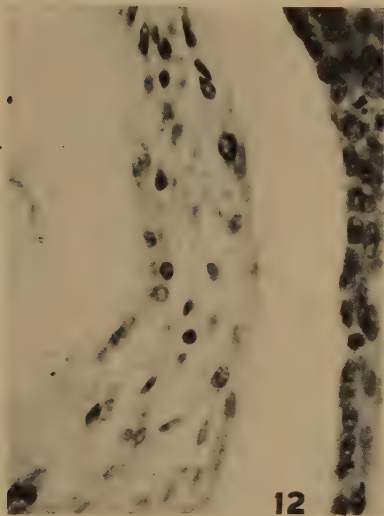
EXPLANATION OF FIGURES

12 Mesoderm of the basal chorion. Most of the cells are fusiform with elongate nuclei containing scattered, finely divided chromatin. Occasional free spherical cells are to be found. Chorionic epithelium to the right with shrinkage space between it and the mesoderm. $\times 600$.

13 Linearly arranged cells (x) with large nuclei which form protoplasmic strands which might be interpreted as evidence of vasculogenesis. $\times 600$.

14 Cellular detail from the core of a villus illustrating stages (1, 2, 3, 4) in the transformation of typical mesodermal cells into free spherical cells with basic cytoplasm and very vesicular nuclei with prominent nucleoli characteristic of hemoblasts. $\times 1340$.

15 An accumulation of free round cells at the tip of a villus suggestive of the appearance of blood islands. Near the lower border of the picture is a column of mesodermal cells suggestive of angiogenesis. $\times 600$.



A CASE OF LEFT POSTRENAL INFERIOR VENA CAVA WITHOUT TRANSPOSITION OF VISCERA

FRANCIS JAMES L. BLASINGAME AND CURTIS H. BURGE

Department of Anatomy, University of Texas, Galveston

TWO FIGURES

Instances of left postrenal inferior vena cava without transposition of viscera are quite unusual, if not rare.¹ One such case found in 1937 in an adult male during the dissection of a cadaver in the anatomical laboratory of the University of Texas is here reported, this case being the only one recorded in this laboratory in which approximately 2000 bodies have been dissected.

CASE REPORT²

The common iliac veins commenced in the usual manner. The left common iliac vein ran upward and medially parallel and lateral to the corresponding artery. The right common iliac vein coursed upward and medially just below and parallel to the right common iliac artery and then passed posterior to the upper end of the left common iliac artery to unite with the left common iliac vein to form the beginning of the inferior vena cava in front of the upper border of the fifth lumbar vertebra, somewhat to its left side (fig. 1).

The inferior vena cava thus formed passed vertically upward for 8 cm. where it was joined on the left side by the two left renal veins. The left internal spermatic vein emptied into the ventral aspect of the inferior vena cava just below

¹See 'Literature Cited.'

²The authors wish to express their appreciation to Prof. H. O. Knight for permission to publish this case and to Professors Knight and John G. Sinclair for their helpful suggestions and criticisms.

the lower left renal vein. This part of the inferior vena cava lay against the bodies of the fifth, fourth and third lumbar vertebrae on the left ganglionated sympathetic chain and left psoas major muscle parallel with and immediately to the left of the abdominal aorta.

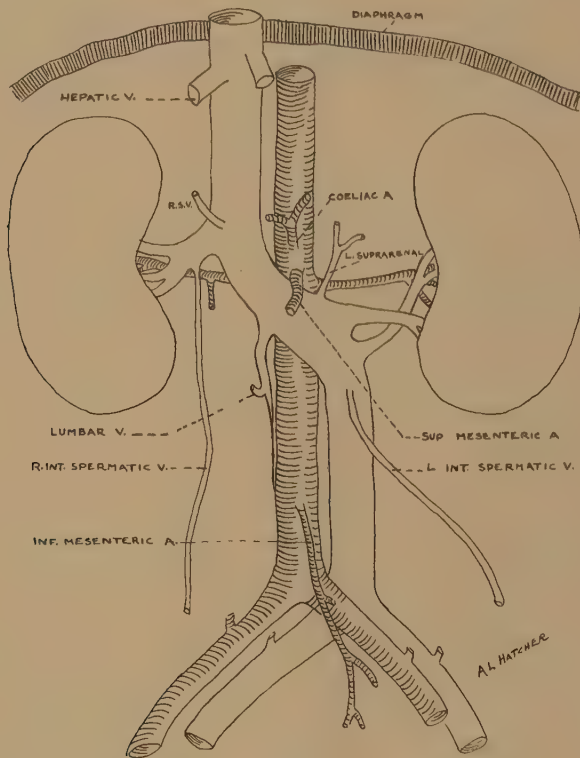


Figure 1

At the level of the third lumbar vertebra, after receiving the left renal veins, the inferior vena cava passed ventral to the abdominal aorta, obliquely upward and to the right, to gain its usual position to the right of the aorta. This oblique portion was 6.5 cm. in length, while the remainder, extending vertically to the right atrium, measured 9.5 cm.

Other tributaries consisted of a left supra-renal vein from above on the left and a good sized lumbar vein which coursed

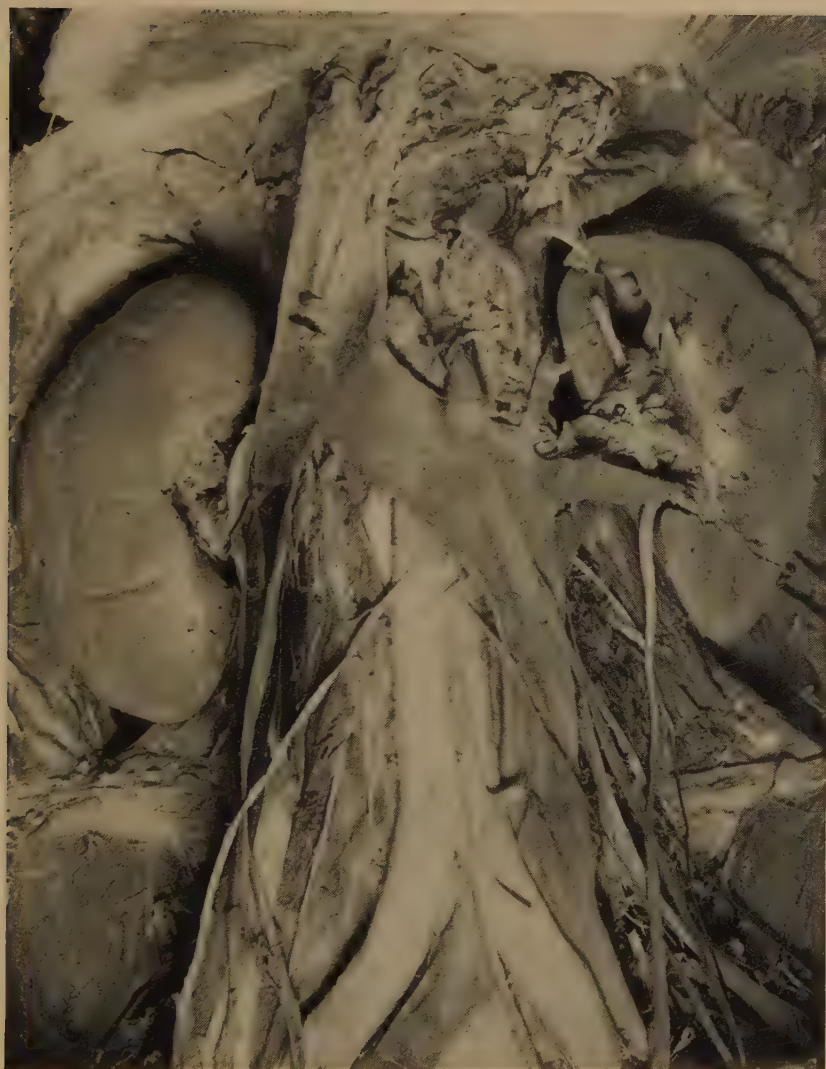


Figure 2

horizontally around the right side of the third lumbar vertebra and then turned upward at a right angle along the right side of the aorta to join the oblique part of the inferior vena cava from below. This latter tributary to the inferior vena cava was joined below and medially opposite the right angle bend by a delicate vein and band of fibrous tissue which lay on the front of the fourth lumbar vertebra and extended downward and medially behind the aorta. Below this level this delicate vein and the fibrous band became so attenuated that it could not be followed any farther inferiorly. Apparently, this delicate vein and band of fibrous tissue had served to fix or anchor this lumbar vein and produce the sharp bend in its course.

At the point where the oblique portion of the inferior vena cava become continuous with the vertical portion, it was joined by the right renal vein. The right renal had received farther to the right the corresponding internal spermatic vein.

Above this level the inferior vena cava was normal. The veins in the chest showed no abnormality. There was no transposition of the viscera. The spinal column possessed only normal curvatures.

The abdominal aorta, while in its normal position above, gradually assumed a position farther to the right than normal until at its bifurcation it lay slightly to the right of the midline of the lower part of the body of the fourth lumbar vertebra. The aorta was normal in size. No reason for this displacement to the right was apparent.

There were two left renal veins which exhibited an unusual twisting so that the vein which arose nearer the lower pole of the kidney, after crossing ventral to the vein which arose nearer the upper pole, joined the inferior vena cava at a higher level than the vein which arose near the upper pole (fig. 1).

COMMENT

The position of the post-renal portion of the inferior vena cava on the left side of the aorta in this case can be explained by the persistence of the left supracardinal vein (para-ureteric vein of Reagan, '26-'27) and its connections to the left sub-supracardinal and subcardinal anastomoses cranially and postcardinal anastomosis caudally and the disappearance of the right supracardinal vein (Arey).

The fact that the lower part of the abdominal aorta in this case, for some obscure reason, was located farther toward the right side than normal may explain the presence of this anomaly. The right embryonic veins usually persist because they represent the more direct route to the heart, thus offering less resistance to the flow of blood. With pressure on these right embryonic veins by the abdominal aorta, resistance was increased beyond that of the left side and resulted in obliteration of the right embryonic veins and the persistence of the corresponding left veins. This view is substantiated by the presence of a delicate vein and fibrous band which we believe to be remnants of the channels which would have formed a normal inferior vena cava, since the vein and fibrous band were located on the right side of the vertebral column behind the aorta and were connected with the oblique part of the inferior vena cava.

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THE RELATIONSHIPS OF THE EPITHELIAL COMPONENTS OF THE PITUITARY GLAND OF THE RABBIT AND CAT

ALDEN B. DAWSON

Biological Laboratories, Harvard University

TWO PLATES (SEVENTEEN FIGURES)

During the course of the examination of a large number of differentially stained, serial sections of the rabbit and cat (Friedgood and Dawson, '37) the indefiniteness of certain of the relationships between the pars anterior proper, pars intermedia and pars tuberalis became evident. In the rabbit neither the pars intermedia nor the pars tuberalis are completely and sharply segregated from the anterior lobe proper but each may invade it to a greater or less extent. In the cat the pars intermedia is for the most part rather clearly limited from the pars anterior except in a restricted zone near the stalk. On the other hand the pars tuberalis invades the anterior lobe to a much greater extent. This encroachment of one part on the other is of little significance from the standpoint of the general organization of the pituitary gland but is of great importance in studies dealing with the quantitative relationships of the three classes of cells commonly recognized in the pars anterior. The cells of the pars tuberalis proper are chiefly chromophobes with relatively little infiltration of basophiles and acidophiles while the pars intermedia is made up primarily of basophiles. In many studies in which differential cell counts on the pars anterior have been made either no mention is made of the lack of clear limitations of the three epithelial components or the condition is dismissed as of little quantitative significance. The validity of the procedure is questionable.

The present observations are based on pituitaries which have been sectioned either frontally or sagittally, and differentially stained by a slight modification of Heidenhain's 'azan' modification of Mallory's triple connective tissue stain, following fixation in a saturated solution of corrosive sublimate in physiological saline, 9 parts, and formalin, 1 part (Dawson and Friedgood, '37). In the pars anterior proper the acidophiles are either a deep red or bright orange, the basophiles deep blue or light blue, and the chromophobes are colorless or a light pink. The specific cells of the intermedia are deep blue and light blue with scattered chromophobes, and in the rabbit the epithelium of the irregular, cyst-like spaces, sometimes ciliated, representing vestiges of the residual lumen, stains similarly to the chromophobes. In the tuberalis proper the cells react as chromophobes and colloid, when present, stains a deep blue. However, in the region more closely associated with the pars anterior proper the reaction becomes variable, some times chromophobes predominate, at other times basophiles are almost exclusively present or there may be a mixture of chromophobes and basophiles.

THE TUBERALIS OF THE RABBIT

In the rabbit, Wolfe, Phelps and Cleveland ('34) recognized the peculiar concentration of basophiles (cells of type III) of the anterior pituitary in a small area on the periphery of the gland, on the antero-ventral surface directly opposite the posterior lobe. According to their description basophiles were found in this area almost to the exclusion of acidophiles (cells of type I), although a large number of chromophobes (cells of type IV) were present. They also noted that this area was well indicated on the antero-ventral surface of the fresh unfixed gland near the upper pole, where it appeared to be much redder in color than the remaining portion of the gland. In the region closer to the lower pole of the gland this isolated area gradually blended into the more general area of the anterior lobe where all three types of cells were found freely mixed. However, they felt that the cells of this special

area made up only a very small percentage of the total number of basophilic cells in the pars anterior.

Their observations were based on frontal sections. When serial sagittal sections are studied it is seen that this antero-ventral zone is of considerable extent and is morphologically continuous with the pars tuberalis and that the organization of the epithelial elements of the region gradually changes until the typical structure of the tuberalis is attained (fig. 1). In the area which is incorporated in the pars anterior the epithelial cords are more closely packed, being invested by very delicate connective tissue septa, but they gradually become more widely separated and eventually acquire lumina which may contain colloid, giving a vesicular appearance to the tissue, and characteristic of the pars tuberalis.

The complete extent of this zone within the pars anterior and its relationship with typical tuberalis tissue is best seen in sagittal sections (figs. 1, 2, 3); its lateral extent is more readily determined in frontal sections (figs. 8, 9). Its general form is that of a relatively broad tongue of tissue which extends ventrally and caudally from the tuberalis toward the lower end of the pars intermedia, becoming less and less distinctly separated from typical anterior tissue and lying deeper in the gland toward its termination. The typical tuberalis forms an epithelial collar investing the infundibular stalk (figs. 4, 5) and more ventrally assumes the form of a solid cylinder lying anterior to the main body of the gland and sharply separated from the anterior proper by a dense band of connective tissue which frequently contains small bands of smooth muscle running transversely to the sagittal plane (figs. 6, 7). More ventrally it is fused with the anterior lobe (fig. 8) and gradually becomes merged into the special zone of this portion of the gland (fig. 9). This sheet of connective tissue extends deep into the anterior lobe along the dorsal surface of the tongue-like epithelial extension from the tuberalis proper and is accompanied by relatively wide blood vessels which appear to become continuous with the dilated capillaries in the central area of the pars anterior, adjoining

the region of the residual cleft (Wolfe, Phelps and Cleveland, '34).

Warbritton and McKenzie ('37) describe the pars anterior of the ewe as differentiated into a central core and a peripheral zone but do not indicate the relative proportions of the two regions. They also point out that the histological structure of the core is very similar to that of the tuberalis region and that in fresh tissue a medial sagittal section shows a vascular area radiating from the anterior tuberal part through the central part of the pars anterior.

The morphological relationships of the tuberalis proper and the antero-ventral area of the pars anterior proper strongly suggest that this special zone might be regarded as an integral part of the tuberalis complex (derivatives of the lateral lobes) and this view is supported by the relationships clearly described by Atwell ('18, figs. 28, 31, 35) in the developing gland of the rabbit. Dorsally, in the developing gland, the pars tuberalis is separated from the main body of the gland by a wide fossa which is filled with connective tissue. Later the fossa becomes greatly reduced and the connective tissue it contains is compressed. As Atwell ('18) points out this imprisoned connective tissue is a landmark of greatest importance since it serves to demark sharply the pars tuberalis from the anterior end of the pars intermedia.

Furthermore as shown in his illustrations the connective tissue of the fossa extends ventro-caudally beyond the intermedia, into the main glandular mass. The tissue so separated and continuous anteriorly with the pars tuberalis is apparently derived from the lateral lobes which also give rise to the pars tuberalis proper. The portion of each lateral lobe, between the brain wall and the oral epithelial stalk, is deeply constricted off from the remainder of the gland and eventually forms a temporary cortex limited to the nasal end of the anterior lobe proper. Atwell ('18), however, believes that in the rabbit the lateral lobes do not form a cortex for the entire anterior lobe and that the localized cortex as seen in a 17- and 18-day embryo does not persist long in this position and eventually disappears. Later in development, the

point of attachment of the epithelial stalk shifts from the ventral side to the nasal end of the anterior lobe and comes very close to the attachment of the pars tuberalis. This is due to the bending of the gland to form the fossa, to the approximation of the lateral lobes to the brain wall in the formation of the pars tuberalis and to a rapid growth of the glandular anterior lobe proper. In the differential growth which carries the stalk anteriorly it appears possible that the cortical area of the nasal end of the gland does not disappear as Atwell suggests but is overgrown to a considerable extent by the anterior lobe proper and becomes partially embedded in it. Accordingly the special zone on the antero-ventral surface of the gland, sharply delimited dorso-caudally by the persisting connective tissue of the embryonic fossa and merging gradually into the pars tuberalis proper, should possibly be regarded as a persisting cortex derived from the lateral lobes, even though it does not occupy a true cortical position throughout its entire extent.

Since the embryonic history of this special zone, lying on the median antero-ventral surface of the anterior lobe, is not clear it seems unwise to refer to it as a cortex and it is proposed, on the basis of its morphological relation to the pars tuberalis, to designate it as the zona tuberalis.

. PARS INTERMEDIA OF THE RABBIT

In the adult rabbit the residual lumen persists to varying degrees. In some pituitaries it is seen as a continuous slit of considerable extent, in others it is represented by a varying number of closed vesicles which are frequently ciliated and may contain a basophilic colloid. In the more central region of the gland the pars intermedia is usually sharply limited from the pars anterior proper and there is little intermingling of cellular elements of these two components of the gland even though they are in intimate contact with each other, following the reduction or partial disappearance of the residual lumen. However, more peripherally at the region where the intermediate tissue, formed with the dorsal wall

of the hypophyseal sac, retains its original embryonic continuity with the portion giving rise to the pars anterior there is a considerable intermingling of anterior and intermediate tissue (figs. 1, 2, 9). This intermingling occurs in a narrowly restricted zone in the ventral and ventro-lateral margins of the intermedia but dorso-laterally it is quite extensive. Embryologically in this dorsal region processes of the pars intermedia grow up and almost, or completely surround the portion of the neural lobe with which they are in contact (Atwell, '18) so that in the adult pituitary only the more caudal portion of the posterior lobe is fully exposed on the surface of the gland.

Laterally the tissue of the pars anterior also accompanies these dorsal intermediate processes (figs. 5, 6, 7) so that the narrower, more dorsal portion of the posterior lobe lies embedded in the pars intermedia which in turn lies in a depression of the pars anterior proper. In this entire zone the basophilic cells of intermedia are not sharply delimited but extend as irregular clusters into the anterior tissue, the basophilic infiltration being most extensive in the antero-ventral region (figs. 1, 2). Accordingly in the rabbit there is a relatively large area of the pars anterior in which it is very difficult to distinguish the basophiles infiltrated from the pars intermedia from the true, deeply basophilic cells of the anterior proper as clustered basophiles, presumably of intermediate origin, become isolated and merge imperceptibly into the characteristic mosaic of basophiles, chromophobes and acidophiles of the anterior tissue.

Although the pars intermedia is very closely associated with the posterior lobe it does not invade it to a marked degree. At the periphery of the gland there is a slight extension of the basophilic cells at the edge of the intermedia into the posterior tissue but more centrally this does not occur with any regularity. However there are occasional areas where apparently a true infiltration has occurred so that isolated clusters of basophilic cells may be found deep in the neural tissue. In other instances, an appearance of infiltration is

given in sections by the irregularities in the contours of the two opposing tissues.

Atwell ('18) has noted localized areas of contact or connections between the tissues of these two parts but he regarded them as due to the active growth of processes of the neural lobe into the intermediate part and does not interpret them as evidences of invasions of the neural lobe by cell masses from the intermedia (Herring, '08). It appears possible that such connections as occur in the embryo may provide the means for a mutual invasion by elements of the two parts.

PARS TUBERALIS OF THE CAT

The general morphological relations of pars tuberalis and the pars anterior of the pituitary of the cat are strikingly similar to those of the rabbit but there is no definite landmark such as the connective tissue of the fossa to sharply demark the pars tuberalis from the intermedia and to delimit dorsally the extension of the so-called zona tuberalis into the pars anterior.

The tissue of the tuberalis proper has a conspicuous vesicular appearance due to the accumulation of colloid in the lumina of the cords and the wide separation of the epithelial units by loose connective tissue. It appears as relatively thin sheets investing the infundibular stalk (fig. 10) but is greatly thickened at the regions where it joins the median antero-dorsal margin of the pars anterior (fig. 11) i.e., in the region of saccular eminence of the tuber cinereum (Tilney, '13).

At this point the tissue is distinctly separated into a peripheral, loose, vesicular zone and a central, compact zone. In the compact zone the interstitial connective tissue is greatly reduced and the epithelial cords rarely contain colloid. This central area becomes continuous with the anterior lobe, forming an irregular zone which occupies the median, antero-ventral region of the pars anterior and extends centrally to the pars intermedia (figs. 12 to 17). This central mass

is accompanied by short ventral processes of typical tuberalis tissue which lie on the surface of the anterior lobe just lateral to the compact tissue (figs. 14, 15).

This extension of the compact portion of the tuberalis area into the anterior lobe seems to be homologous with the similar region described in the rabbit which has been designated, *zona tuberalis*. In the cat the typical tuberalis tissue is sharply delimited from the *zona tuberalis* while in the rabbit one gradually merged into the other. On the other hand the *zona tuberalis* is more localized in the anterior lobe of the rabbit. In the cat it is infiltrated by scattered acidophile cells and is frequently broken into irregular cords and masses by invasions of typical pars anterior tissue (figs. 15, 16, 17). Histologically it is identical with the *zona tuberalis* of the rabbit, being composed predominately of chromophobic and basophilic cells.

As in the rabbit this region, characterized by the marked restriction of acidophiles, has not been recognized in studies of the embryology of the gland in the cat (Brahms, '32) and no additional information on its developmental history can be gained from this species.

THE PARS INTERMEDIA OF THE CAT

The pars intermedia of the cat completely invests the posterior lobe. It fuses with the pars tuberalis proper and the *zona tuberalis* in the region of the infundibular stalk without any sharp line of demarcation (figs. 10, 11, 12). Brahms ('32) on the other hand, has described the pars tuberalis as distinctly separated from the pars intermedia by connective tissue as in the rabbit. Our observations agree with the earlier description of Tilney ('13). However the pars intermedia except for a restricted zone near the stalk is completely separated from the pars anterior proper by the persistence of a well-defined residual lumen. Accordingly there is no problem of infiltration of basophilic cells of the intermedia into the anterior lobe proper such as occurs in the rabbit.

DISCUSSION AND SUMMARY

Attention is called to the presence in the pituitary gland of the rabbit and cat of a specialized zone of tissue which occupies the median antero-ventral region of the pars anterior proper and is continuous antero-dorsally with the thickened portion of the pars tuberalis associated with the saccular eminence of the tuber cinereum. Owing to its morphological relation to the pars tuberalis it has been designated as the zona tuberalis of the pars anterior.

This region is characterized primarily by the marked restriction of acidophiles and the predominance of basophiles and chromophobes. In addition this zone exhibits great shifts in cell population being at times almost exclusively occupied by basophiles, and at others, by chromophobes. These changes are correlated with certain phases of the reproductive cycle and are also induced by certain experimental procedures. In the castrated female rabbit the area is almost exclusively basophilic (Smith, Severinghaus and Leonard, '33) and in the adrenalectomized cat the basophiles may practically disappear leaving the tissue predominately chromophobic. These changes occur in all of the compact tissue of the zona tuberalis but rarely invade the typical vesicular tuberalis tissue to any extent. The extreme lability of the tissue of this area so far as basophilic and chromophobic cells are concerned stands in marked contrast with the behavior in the typical pars anterior where the changes in the population of these cells are much less marked.

The functional significance of the cells of the zona tuberalis is not clear but the distribution of the cells in this region cannot be ignored in making differential counts of the cellular constituents of the anterior pituitary of either the rabbit or cat. In addition, the evaluation of the basophilic population of the anterior lobe of the rabbit is further complicated by a considerable intermingling of the cells of the pars intermedia with those of the pars anterior, especially in the antero-dorsal region where the intermedia grows up to invest the adjoining portion of the posterior lobe.

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PLATES

EXPLANATION OF PLATES

All figures, both of the rabbit and cat, were outlined at the same magnification and the distribution of the various parts indicated as follows: pars anterior proper, fine stipple; pars intermedia, solid black; pars tuberalis proper, large open circles; zona tuberalis, large solid dots; pars posterior, irregular lines.

All sections were cut 4μ thick and differentially stained, following fixation in corrosive sublimate-formalin by a slight modification of Heidenhain's 'azan' stain.

Plate 1 illustrates the relationships in the pituitary of the rabbit, plate 2 in the cat.

PLATE 1

EXPLANATION OF FIGURES

1 Sagittal section of the rabbit pituitary. The region of the stalk has been reconstructed to a slight extent as this is usually distorted in the removal of the gland and is seldom cut in a sagittal plane. F indicates the connective tissue of the embryonic fossa which separates the pars tuberalis from the pars intermedia and extends into the pars anterior along the dorsal surface of the zona tuberalis. Note the diffusion ventrally of intermedia tissue of the antero-lateral region into the pars anterior. The numbered lines (4 to 9) show the approximate levels from which the frontal sections (figs. 4 to 9) were drawn. The meningeal relationships are not shown.

2 Parasagittal section slightly lateral to the infundibular stalk and passing through the more lateral portion of the antero-dorsal extension of the pars intermedia.

3 A similar section lateral to the antero-dorsal extension of the pars intermedia and passing through the lateral margin of the zona tuberalis.

4 A frontal section through the stalk cutting the dorsal extension of the thickened pars tuberalis in the region of the saccular eminence and passing through the recessus tuberalis.

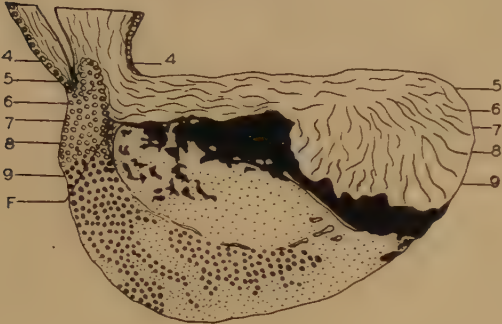
5 A frontal section showing the relationships of the several parts as the stalk joins the body of the gland. In this and the succeeding four sections the indefiniteness of the boundary zone between pars intermedia and pars anterior is shown.

6 A frontal section showing the cylindrical portion of the tuberalis, medial and anterior to the body of the gland.

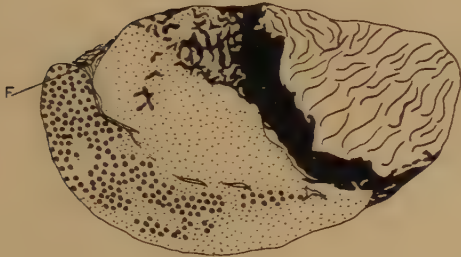
7 A frontal section showing the gradual transition from the pars tuberalis proper to zona tuberalis and the separation from the body of the gland by the connective tissue of the fossa (F).

8 A frontal section showing the zona tuberalis embodied in the pars anterior and passing through the ventro-caudal extension of the fossa.

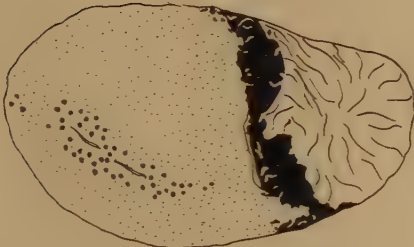
9 A frontal section showing the extent of the zona tuberalis at this level and the infiltration into it of typical pars anterior tissue.



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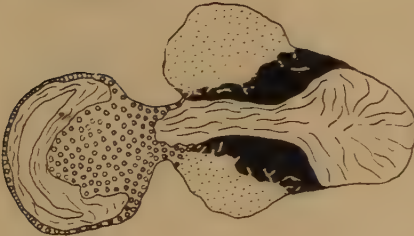
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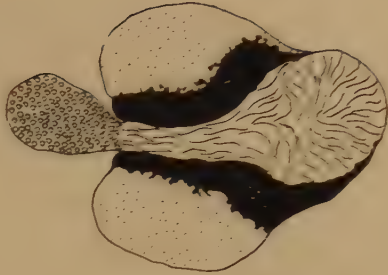
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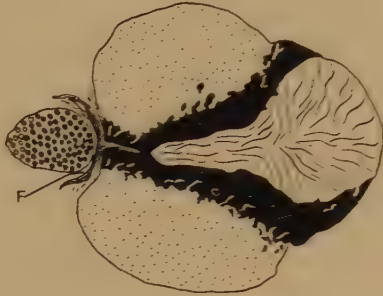
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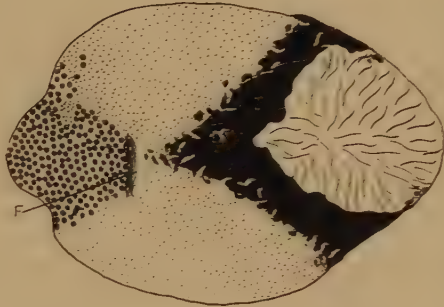
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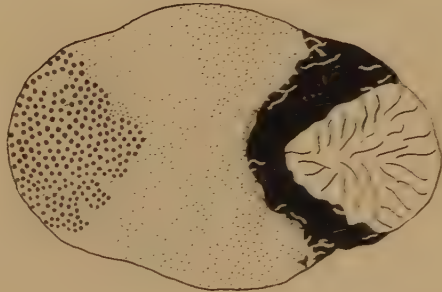
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PLATE 2

EXPLANATION OF FIGURES

In these figures the pars anterior is shown embracing the posterior lobe but not completely surrounding it as it sometimes does. In the adult cat the posterior margin of the typical anterior lobe frequently ends abruptly and is continued as a thin layer of connective tissue, with scattered epithelial cells. It extends caudally to join the intermedia lying on the posterior surface of the infundibulum and thereby encloses the residual lumen. This thin layer was removed along the capsule of the gland.

10 Frontal section passing through the stalk and showing the absence of any sharp demarcation of pars tuberalis, intermedia and anterior.

11 Frontal section (184 μ ventral to the level of fig. 10) passing through the tuberalis in the thickened region associated with the saccular eminence of the tuber cinereum and showing the sharp segregation of the tissue into a loose vesicular, peripheral and a compact, central zone.

12 Frontal section (92 μ ventral to the level of fig. 11) showing a more intimate association of the compact tissue (zona tuberalis) with the pars anterior.

13 Frontal section (120 μ ventral to the level of fig. 12) showing the zona tuberalis incorporated into the median anterior region of the pars anterior with ventral extensions of typical pars tuberalis tissue lying laterally. These ventral extensions may also be seen in figures 14 and 15.

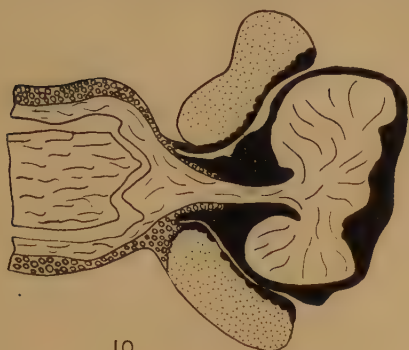
14 Frontal section (160 μ ventral to the level of fig. 13).

15 Frontal section (260 μ ventral to the level of fig. 14).

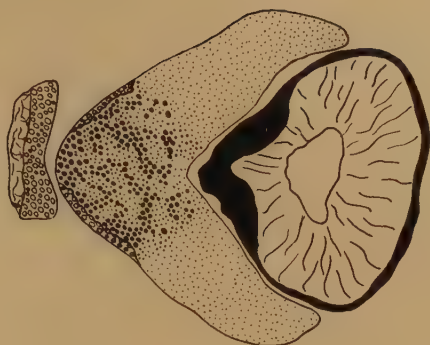
16 Frontal section (228 μ ventral to the level of fig. 15).

17 Frontal section (240 μ ventral to the level of fig. 16).

In these four figures the extent of the tissue of the zona tuberalis and the degree of infiltration by the tissue of the typical pars anterior are illustrated.



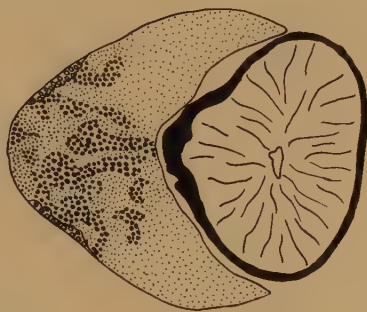
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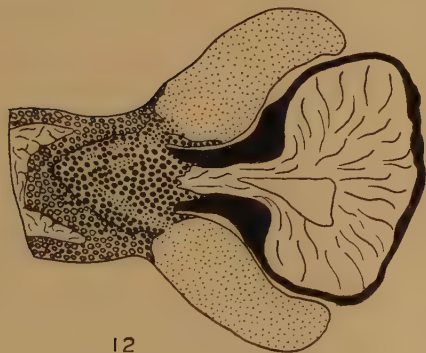
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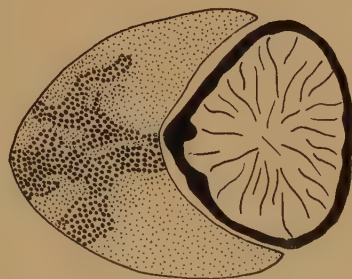
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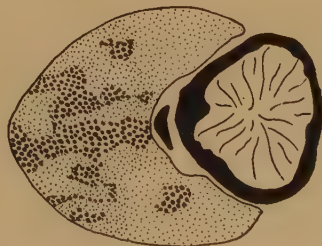
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CYCLIC CHANGES IN LYMPHATIC NODULES

ELEANOR A. CONWAY

*Department of Anatomy, The University of Chicago*¹

THREE PLATES (EIGHT FIGURES)

Although there is a general agreement that the lymphatic tissue is composed of a reticular stroma and lymphocytes, the nature and functions of its nodules are presented in the voluminous literature as a maze of contradictory experimental and descriptive data, opinions and theories. Much of the disagreement on the nature of the nodules centers about the opposing views of Flemming (1885), Downey and Weidenreich ('12), and Jordan ('35) on the one hand and Hellman ('21, '30) on the other; the former believe that the nodules are centers of proliferation of lymphocytes and the latter holds that they are foci for the destruction of these cells. With the exception of Wätjen ('25), Maximow ('27, '28), Taliaferro and Cannon ('36), Bloom ('37), and Taliaferro and Mulligan ('37), there are practically no compromise views in the literature.

Flemming's 'germinal center' nodule consists of an outer dark-staining zone of small lymphocytes surrounding a paler-staining center of medium and large lymphocytes, scattered reticular cells and a few macrophages, occasionally with debris of dead cells in their cytoplasm. The prominent feature of the central portion is the great number of lymphocytes in mitosis. Hellman's 'reaction center' nodules also consist of an outer zone of small lymphocytes while the central lighter-staining area contains few normal lymphocytes, scattered reticular cells, numerous macrophages and many dead and dying lymphocytes.

¹ This investigation was aided by a grant from the Rockefeller Foundation to The University of Chicago.

The purpose of this paper is to explain, on the basis of experiment, the contradictory opinions on the structure and nature of the nodules of the lymphatic tissue. A reëvaluation of the confused and at times contradictory terminology is necessary, as well as a correlation between the structure of the lymphatic nodule and its function. There is no clear concept of the relationship of the lymphatic nodule to the rest of the lymphatic tissue, nor is there a consensus of opinion on the structure or the significance of the nodule. This confusion is due in part to the failure to study the lymphatic tissue with careful cytological methods under experimental conditions of hyperplasia as well as of hypoplasia and regeneration.

The chief criticism of previous approaches to this problem is the failure to realize that the lymphatic tissue as a whole, as well as the lymphatic nodule, is very labile and that its appearance at any time is only a reflection of its function at that particular time. The structure of both the lymphatic tissue and its nodules varies within very wide limits depending on the intensity and nature of the stimulus and on the time at which the lymphatic tissue is examined in relation to the introduction of the stimulus.

The adherents of any one theory of function seem to disregard entirely the constant variations in the structure of the lymphatic nodule which do not agree with their particular theory. Hellman and his school for example, even though presenting figures of lymphocytopoietic, that is germinal centers many times throughout their numerous papers, insist that the nodule is not a site of formation of lymphocytes but of their death. Their reports were based at first on human material obtained at autopsy from patients who had succumbed to various infectious diseases; they represent, almost without exception, terminal stages of the infections and intoxications. These findings tend to emphasize degenerative changes rather than those associated with successful defense and repair. All of the later experimental studies on lymphatic tissue by this school, in which a wide variety of stimuli were used, are claimed to substantiate the original conten-

tion of Hellman ('21) that the lymphatic nodule is a reaction center, not a lymphocytopoietic center.

It is obvious that the function of the lymphatic nodule in the adult as a 'Keimezentrum' of Flemming cannot be denied, as Ehrlich has done ('29 b), because it is not present in the embryo. On the other hand it is equally fallacious to claim lymphocytopoiesis as the sole function of the nodule, when it can be demonstrated so easily in infection with certain bacteria and parasites, as well as with other noxious agents, that the lymphocytes of the lymphatic nodules may be the first to be destroyed by the toxic agents (Epstein, '29; Wätjen, '25; Ehrlich, '29 c, '31; Taliaferro and Cannon, '36). But even in the latest papers of Hellman's students, although their verbal descriptions and their photographs show the presence of 'germinal' lymphatic nodules, the conclusion is reached that the nodule is not a site of production of lymphocytes but a reaction center in the sense of Hellman (Sjövall and Sjövall, '30; Hellman and White, '30; Sjövall, '36 and Glimstedt, '36).

A study of the early stages of the infection of rabbits and guinea pigs with *B. monocytogenes* shows many variations in the lymphatic nodules which are of particular interest in view of this controversy concerning their structure as well as their function (Conway, '37). The difference between this and previous studies is the fact that these animals were examined at close intervals, especially during the early stages of the infection. This emphasizes the conclusion that the effect of the introduction of a stimulus into an animal must be followed from the time of the injection at close intervals through the initial reaction, the peak and the recovery periods, or until the animal dies. If one waits until the animal succumbs to or recovers from an infection and then, from the appearance of the lymphatic tissue at that time, postulates a theory as to its function during the course of the infection, the resulting conclusion, based on incomplete data, must be inadequate.

None of the previous descriptions of the supposedly definitive structure of the lymphatic tissue or of the nodule account

for all of the variations in them as seen in this study of the close interval stages of infection with *B. monocytogenes*. This agent not only calls forth a production of lymphocytes and monocytes but also depletes both the diffuse and the nodular lymphatic tissue of its free cells. In addition, in the phase of regeneration following this extreme depletion one can follow all stages in the development of the nodular lymphatic tissue. In the light of these and of the previous experiments it is necessary to redefine the terms used in describing the various parts of the lymphatic tissue.

If one follows the classification of Aschoff ('26), in which he distinguishes lymphatic tissue from lymphoid by the absence of 'germinal centers' in the latter, one would have to consider the cortex of the lymph node as lymphatic tissue when 'germinal centers' were present or lymphoid if they were absent. In adopting this classification, Ehrlich ('29 a) has done just this and speaks of lymphatic tissue as only the 'germinal centers,' and further complicates Aschoff's division by stating that everywhere the 'germinal centers' are surrounded by lymphoid tissue. This division of the cortex of a lymph node into lymphatic and lymphoid tissue is essentially incorrect, since it can be shown that the nodule is not a permanent structure but may appear and disappear and reappear at any point throughout the cortex and medulla of the lymph node.

Lymphatic tissue may be 1) dense diffuse, as found in the cortex of a lymph node or in the periarterial sheaths in the spleen, 2) loose, as found in the medullary portions of a lymph node, 3) nodular, in both the loose and the dense diffuse lymphatic tissue. These three variations are interchangeable, one transforming into any of the others, depending on the nature of the stimulus. The basic framework of these three divisions is the same and is composed of a network of reticular fibers closely associated with reticular cells. In all three types the free cells are the same and consist of all sizes of lymphocytes and a smaller and variable number of free macrophages. In any section of a normal lymph node or spleen three types of lymphocytes can usually be distinguished: 1) Large lymphocytes are scattered singly among

the greater numbers of medium and small lymphocytes. These large lymphocytes have a broad band of deeply basophil cytoplasm surrounding the characteristic, large, slightly eccentric nucleus. The nuclear membrane is coarse and the several chromatin particles are widely separated by a large amount of nuclear sap; one or several, large, angular, acidophil nucleoli are always very prominent. 2) The medium-sized lymphocytes are somewhat smaller; the chromatin of the nucleus is slightly denser but the one or several acidophil nucleoli are always present. The cytoplasm is basophil and less in amount relatively, than in the large lymphocytes. 3) The small lymphocytes contain a relatively large nucleus; its compact and large chromatin particles tend to obscure the large nucleolus. Only a thin layer of cytoplasm surrounds the nucleus and accumulates in somewhat greater amount around its indentation.

In any complete cytological analysis of any variety of lymphatic tissue, the nature of the free cells of this tissue cannot be disregarded as Hellman has done. This is especially true in delimiting nodular lymphatic tissue from either dense diffuse or loose lymphatic tissue. If the same criteria for identifying the free cells of the diffuse tissue are applied to the cells of the nodular lymphatic tissue, the lymphocyte nature of the cells is as obvious in the one as in the other. Hellman's reference to the confused literature is insufficient basis for his failing to identify them as lymphocytes when they appear in the nodular lymphatic tissue. Glimstedt ('36), a pupil of Hellman, describes 'germinal center cells' but does not admit that they are medium or large lymphocytes and hence concludes that the nodule is not a 'germinal center.' The photomicrographs illustrating this paper, permit the ready identification of the cells in the centers of these nodules as various sizes of lymphocytes. (See figs. 36 and 41 of Glimstedt.)

MATERIAL AND OBSERVATIONS

A series of rabbits, infected with portions of the same cultures of *B. monocytogenes*, were killed at close intervals from the time of the injection until monocytes were present in great numbers in the peripheral blood. A larger group of guinea pigs was similarly treated. A detailed report of the methods as well as the microscopical observations, especially as they relate to the origin and function of the monocyte, are published elsewhere (Conway, '37). Only those findings are described here which deal with the changes in the nodular lymphatic tissue of the mesenteric lymph node and the spleen. This work was carried out in the laboratory of Dr. William Bloom.

Mesenteric lymph nodes

One of the most striking observations in the course of this infection in both rabbit and guinea pig is the marked change in size of the mesenteric lymph node. During the first 24 hours of the infection this node increases in size and at the same time the periarterial lymphatic tissue of the spleen becomes more extensive. This initial hyperplasia is associated at first with an intensive lymphocytopoietic activity, and, by the end of the first day, monocytopoiesis also occurs in both the mesenteric lymph node and in the spleen. This occurs before the appearance of an increased number of monocytes in the peripheral blood.

In the succeeding 24 hours the mesenteric lymph node and the white pulp of the spleen become progressively depleted of lymphocytes, despite an extensive concomitant formation of lymphocytes in the nodular lymphatic tissue in both situations. This depletion is so marked on the third day that the mesenteric lymph node in both rabbit and guinea pig is less than half the size of the nodes in the uninfected animals. This hypoplasia is characterized by an almost complete disappearance, by migration, of lymphocytes from the entire node without, at first, a compensating production of new lymphocytes.

In the guinea pig this hypoplasia, as found on the third day, is followed by a progressive increase in size to the seventh day. At $3\frac{1}{2}$ days a new formation of lymphocytes begins by a transformation of reticular cells of the cortex; it is evidenced by the many intermediate stages between reticular cells and large and medium lymphocytes present at this time. This same process also occurs in the spleen. Then the lymphocytes begin to multiply rapidly by mitosis so that at 4 days numerous small nodular areas of lymphocytopoiesis are present throughout the node. Seven days after the injection of *B. monocytopogenes* this lymphocytopoiesis is so extensive throughout the nodular lymphatic tissue that the mesenteric lymph node has increased to more than twice the size of the node in the uninfected animal. This hyperplasia of lymphatic tissue following the peak of the monocytosis in the peripheral blood is also present in the spleen.

The mesenteric lymph node of normal, young adult rabbits and guinea pigs usually presents, in section, a fairly uniform microscopic appearance with the demarcation between cortex and medulla relatively sharp. The lymphatic nodules, varying somewhat in size and character, are confined for the most part, to the outer cortical margins with occasionally two or three in the cortico-medullary region. The remainder of the cortex is a fairly uniform mass of medium-sized and small lymphocytes with scattered large basophil lymphocytes and inconspicuous reticular cells. The subcapsular sinus in many places is almost free of lymphocytes and shows little or no distention. The medullary region is usually devoid of lymphatic nodules, its cords and sinuses moderately filled with varying-sized lymphocytes; a few free macrophages occur in the sinuses.

Very shortly after the intravenous injection of the organism (1 hour) these lymph nodes show an extensive movement of the lymphocytes from the outer cortical zone into large irregular masses at the cortico-medullary junction. At the same time some of the nodules in the outer cortical zone have pale-staining centers with many mitotic figures in the lympho-

cytes and dark-staining peripheral areas of small lymphocytes. The number of lymphocytes in mitosis in the extranodular lymphatic tissue is very small in comparison with the great number in the nodules.

The migration of lymphocytes continues to such a degree that 2 hours later, that is 3 hours after the injection, the fixed cells of the reticulum are very prominent in many places in the cortex. During this period the subcapsular and peritrabecular sinuses can be seen to be receiving numerous lymphocytes from the neighboring nodules as well as from the diffuse lymphatic tissue. In addition, at this time only a few of the pale-staining central areas of the nodules in the outer cortical zone are lymphocytopoietic. Most of these areas, which might now be called 'reaction centers' contain very few lymphocytes, their reticular cells are prominent, and in many of them, macrophages are numerous (plate 2, B). Some of these latter cells contain debris of dead cells and some yellow-green waste pigment.

The cortical areas of lymphocyte proliferation appear to be exhausted first; this depletion is compensated by an extensive formation of new areas of proliferation at the corticomedullary junction, increasing in amount during the next 12 hours. In this region there are now numerous areas of active lymphocytic proliferation, presumably of varying ages of activity. Some of the smaller of these areas of proliferation appear simply as isolated masses of densely packed, rapidly dividing, medium-sized lymphocytes in the diffuse lymphatic tissue of this region; these smaller ones can be described as 'bare' germinal centers (plate 1, A, B). Other larger but otherwise similar foci of proliferation are surrounded by a smaller or larger area of densely packed small lymphocytes arranged concentrically around the lighter area (plate 2, A). This is a significant observation in its bearing on the nature of lymphatic nodules. The character of these proliferating cells as medium-sized lymphocytes must be stressed; there is nothing indefinite about their nature. That the medium-sized lymphocytes are diving in these areas is

certain, since, of the numerous mitoses in them, many are in prophase. Hence there is no question of an inability to identify them as medium-sized lymphocytes. Glimstedt ('36) pictures nodules similar to these described here, but does not recognize that these cells of the central portions are medium-sized lymphocytes or that the many mitoses present are in lymphocytes.

In later stages, as the activity of the node increases in response to the injection, one can find all stages in the formation of a typical textbook nodule, with its supposedly characteristic outer zone of small lymphocytes and its center of larger cells (plate 2, A). These stages vary from an area containing a few rapidly dividing lymphocytes to larger areas of intense proliferation of these cells, which by centrifugal growth pressure compress the smaller, less active lymphocytes of the surrounding diffuse lymphatic tissue. Thus, the concept that one must have a pre-existing primary nodule with its central arteriole, before one can have a secondary nodule or germinal center does not hold in all cases. In this instance, the diffuse lymphatic tissue surrounding the rapidly increasing area of proliferation only assumes the appearance of the accepted picture of a definitive primary nodule. The new nodules do not of necessity arise about arterioles.

If the lymphocytes migrate very rapidly from the cortex, many of the larger areas of proliferation are not surrounded by so-called primary nodules, their only demarcation from the surrounding tissue being the differences in their cellular constitution and the outstretched reticular fiber stroma of the lymphatic tissue. During these periods of hyperplasia of the nodular lymphatic tissue, sections, stained to demonstrate the reticular fiber network of both cortex and medulla, show the characteristic loose, basket-like arrangement of the fibers in the nodules (plate 3, A, B). On the other hand, in similar preparations of the lymph nodes during the extreme hypoplasia of the third day of the infection, when most of the nodular lymphatic tissue has disappeared, no reticular fiber

network characteristic of nodular lymphatic tissue can be demonstrated (plate 3, C, D). This lack of a permanent arrangement of reticular fibers in patterns thought to be constant for the cortex of the lymph node, emphasizes the extreme degree of lability of the various portions of the lymph node. In addition, this interchangeability of reticular fiber patterns indicates that the arrangement usually described as characteristic for lymphatic nodules is produced by the growth pressure of the rapidly dividing lymphocytes during the formation of a lymphatic nodule.

The frequent appearance of lymphatic nodules in the medullary portion of the lymph node throughout the course of the infection is another variation from the accepted concept of the nature and location of the lymphatic nodule. These areas of lymphocytic proliferation in the medulla are first seen 9 hours after the injection of the organism. Here again, one encounters the same picture described for the cortico-medullary region at an earlier period. Some of the areas of proliferation have coronas of small lymphocytes around them, while others are bare, consisting of a compact mass of medium and large lymphocytes with many in mitosis. The appearance of these lymphatic nodules in the medulla of the lymph node occurs again and again throughout the course of the infection and is especially prominent in the guinea pig during the period of hyperplasia following the depletion of the third day. Since in earlier stages it is quite apparent that the nodules are not confined to the outer cortical zone, but may appear anywhere in the diffuse tissue of the cortex, it is plausible to expect that, under persistent stimulus for lymphocyte production, the nodules would appear in the medulla, because the medullary cords are intramedullary extensions of the diffuse lymphatic tissue of the cortex. For the most part the medullary nodules are found in the cords but occasionally they develop by rapid mitosis of lymphocytes presumably caught in the loose reticular meshes of the sinuses.

Spleen

In the spleen of normal rabbits and guinea pigs there is a fairly sharp demarcation of white pulp from red pulp. The periarterial sheaths appear as relatively diffuse lymphatic tissue, the predominating cell being the small lymphocyte. There are only a few nodules present in the sheaths; some of these contain lymphocytes in mitosis, others are 'reaction centers' with few lymphocytes and prominent reticular cells, while a few nodules contain only small lymphocytes. The splenic cords are fairly prominent and the sinuses are moderately filled with various blood cells and macrophages.

If one examines the spleen of either rabbit or guinea pig at any given time during the course of the infection with *B. monocytogenes*, the picture seen is markedly different from that of the normal. The hypoplasia of the white pulp at 12 hours and again at 48 hours in the guinea pig and at 66 hours in the rabbit should be contrasted with pictures of extreme hyperplasia at 24 hours and at 4 days in the guinea pig and at 18 hours and, to a lesser extent, at 48 hours in the rabbit. These changes in the white pulp of the spleen again emphasize the extreme rapidity with which the lymphatic tissue as a whole is capable of responding to a stimulus. It must be stressed that in as short a time as 24 to 36 hours the periarterial lymphatic tissue of the spleen as well as the diffuse tissue of the cortex of the lymph node, can be practically depleted of its lymphocytes. In both rabbits and guinea pigs the arterial sheaths in the spleen 3 days after the injection were practically negligible. This extreme plasticity of structure of the lymphatic tissue in the spleen, as well as the rapidity with which these changes can take place, does not seem to have been emphasized before.

The changes occurring in the spleen during the first 6 hours after the injection of *B. monocytogenes* are similar to those found in the mesenteric lymph nodes, in which organ there is at this time an extensive movement of lymphocytes from the cortex. That the first reaction in the spleen is also a migration of pre-existing lymphocytes is evidenced by the

marked spreading out of the margins of the white pulp into the red. In the guinea pig this reaction is so intense that at 9 hours after the injection the arterial sheaths are very narrow and lymphocyte-poor, and most of the nodules have pale reticular cell centers. In the rabbit during this same time, this spreading out of the white pulp also occurs but there is in addition a new formation of lymphocytes in the nodules of the sheaths, a process which does not begin until after 12 hours in the guinea pig and reaches its height at 24 hours, as compared to 18 hours in the rabbit.

During this early phase of hyperplasia of the white pulp in both rabbit and guinea pig, as well as in the recovery phase in the latter, many variations from the so-called normal descriptions of the arterial lymphatic sheaths and their nodules can be found, which resemble the changes described for the lymph nodes. That the lymphatic nodules can develop anywhere in the diffuse lymphatic tissue of the sheaths is clearly shown. These nodular areas present many varied pictures; all types are present, from small areas of rapidly dividing medium-sized lymphocytes to large ones consisting of pale-staining centers of large and medium lymphocytes surrounded by a compact mass of the smaller cells of the sheath.

It is obvious that neither a pre-existing 'primary' nodule nor a solid secondary nodule of Ehrlich is necessary for the formation of a lymphocytopoietic center. As in the diffuse lymphatic tissue of the mesenteric lymph node, these 'germinal centers' appear as bare areas of proliferating lymphocytes. All stages of these and of typical 'primary' nodules with 'secondary' nodules can be found in the lymphatic sheaths. Here it can be seen that following extensive lymphocytopoietic activity in any type of 'germinal center,' during which the newly-formed lymphocytes can be seen to be moving into the surrounding lymphatic tissue or into the red pulp as the case may be, this 'germinal center' area becomes depleted of its lymphocytes and now appears as a 'reaction center.' In addition, in some of these pale areas transformations from the fixed reticular cells into lymphocytes may be seen. These are

evidences of a new cycle of lymphocytopoiesis. One can also find nodules which are composed of two or three concentric paler and darker areas, showing that with a persistent stimulus one nodule can pass rapidly through several cycles.

At the height of the initial hyperplasia in the spleen many of the cross sections of the sheaths have two or even three distinct foci of proliferating lymphocytes, each area differing from the others. This difference is found mainly in the size of the new area of proliferating lymphocytes, the cell type being the same, medium-sized lymphocytes with a varying number of large lymphocytes in all of them. The larger areas have a peripheral zone of smaller lymphocytes in which some amoeboid medium lymphocytes can be found, while the smaller foci have no concentric ring of smaller cells, being simply surrounded by the diffuse tissue of the sheath. In both earlier and later stages of the infection one can find nodules made up entirely of medium-sized and large lymphocytes; many of both of these cell types are in mitosis. These nodules are devoid of demarcating peripheral zones of smaller lymphocytes. Here as in the mesenteric lymph node lymphocytes in mitosis can be found in the diffuse lymphatic tissue but the number is far less than in the nodular lymphatic tissue. Some of the sheaths in cross section contain a lighter area made up of several concentric but distinct zones of cells, each representing a different phase of activity. In some of these same sheaths there are typical 'reaction centers' and lymphocytopoietic foci.

DISCUSSION

Lymphatic nodules, called secondary nodules by Hellman, primary nodules by Maximow, solid nodules by Ehrich, are described by them as fairly circumscribed areas of closely-packed small lymphocytes with a central arteriole. They are separated from the surrounding lymphatic tissue by virtue of their density and compactness. This concept of a distinct subdivision of the lymphatic tissue, in which characteristically different portions will develop is insufficient to explain the

changes occurring in lymphatic tissue following the injection of *B. monocytogenes*.

On examining the lymphatic tissue at close intervals in this infection the entire cycle of the origin, changes and disappearance of the nodules can be seen. The previous descriptions of the structure or the function of the lymphatic tissue advanced by Flemming, Hellman, Maximow and others are all inadequate in that each of these describes only a phase or phases of activity and none of them describes the complete sequence of events in a nodule. The lymphatic nodule, as shown here, is a more or less circumscribed, oval or spherical area in the diffuse or loose lymphatic tissue, the characteristic features of which vary according to the nature of the stimulus and length of time of its action. Accordingly the attempted separation of this structure into primary and secondary nodules and conclusions as to its function cannot be made on the basis of its appearance at any one time.

That the lymphatic nodule normally passes through cyclic periods of activity is suggested by some of Flemming's figures and is clearly demonstrated by Maximow ('27). During the height of the active phase of this cycle, according to the latter, a clear separation of a central, paler portion from a darker peripheral zone can be made. On the basis of the number of mitoses in the lymphocytes, it is obvious that, at this time, lymphocytopoiesis is much more active in the center of the nodular area than in the darker peripheral zone or in the surrounding diffuse lymphatic tissue. When the height of this active phase passes, there are few lymphocytes present, the reticular cells are prominent and free macrophages are quite numerous. Throughout the area some dying and dead lymphocytes can be found. Maximow believed that with the subsequent disappearance of the pale central area (by release of growth pressure) the lymphatic nodule passed into the resting phase and either merged with the surrounding tissue or developed a new active phase, the site of its re-appearance being marked by the central arteriole.

Each phase of the cyclic activity in the nodule represents one of the dominant theories of the structure or function of the nodule. Thus the active phase of Maximow's cycle is cytologically a 'germinal center' of Flemming; the end of the active phase and beginning of regression of the pale central area resembles the 'reaction center' of Hellman; while the resting phase is the primary or solid nodule of the literature.

In the course of the infection with *B. monocytogenes*, this cycle of activity in the nodule can be followed in all detail, but it does not suffice to produce enough lymphocytes to meet the demand for them. At many stages in this infection, especially during the periods of initial hyperplasia, the normal cycle of a preexisting lymphatic nodule is interrupted and a new active phase develops within a pale central area which has passed the height of its active phase. In addition, an area of rapidly proliferating lymphocytes (active phase) develops anywhere in the lymphatic tissue of the lymph node or spleen, independently of a pre-existing resting phase nodule, 'primary nodule' or 'solid nodule.' These new nodules are the result of active multiplication of lymphocytes in a given region. They appear as dense areas of proliferating medium-sized lymphocytes which bear no relation to a pre-existing nodule. As they increase in size, by repeated mitotic divisions of the lymphocytes, they may or may not become demarcated from the adjacent diffuse lymphatic tissue by an outer zone of smaller cells. It is obvious that if these new nodules develop in the loose lymphatic tissue they would not be surrounded by a circumscribing zone of smaller lymphocytes. The reticular fiber preparations in all of these developing nodules show that the typical basket-like arrangement of the fibers, as has been described for the nodules, is attained by a spreading apart of the fiber-network by the rapidly dividing lymphocytes of the new nodules. The presence or absence of a bordering region of smaller inactive cells around the area of proliferation thus depends on the density of the lymphatic tissue in which the new nodule appears and perhaps, to a minor extent, on the rapidity with which the lymphocytes leave the region of the new nodule.

In those instances in which the bordering zone of small lymphocytes appears, the sharp demarcation of the proliferating area seems to be due to the radial growth pushing out the cells of the surrounding diffuse lymphatic tissue. Hence in this case the corona of small cells cannot be considered an integral part of the new nodule. In many instances the cells of the outer margins of these active centers can be seen to be actively migrating through the corona; these amoeboid cells are, for the most part, medium-sized lymphocytes.

The appearance in the lymphatic tissue of the lymph node or the spleen, of these new areas of proliferating lymphocytes whether in previously developed nodules or not, emphasizes the lability of the lymphatic tissue. It has been pointed out that in the mesenteric lymph node and in the spleen, the first response to the injection of *B. monocytogenes* is from the free cells in the diffuse lymphatic tissue of these organs. Their rapid mobilization and migration from these areas is quickly compensated by an intense new formation of lymphocytes, first in the pre-existing nodules and then in the newly formed nodules and to a much less degree in the diffuse lymphatic tissue. That the lymphatic nodule is a focus of rapid proliferation of lymphocytes is clearly proved on the basis of this material. In the nodular lymphatic tissue of both spleen and mesenteric lymph node typical 'reaction centers' appear only after an intensive lymphocytopoietic activity in the nodules. Throughout the course of the infection all stages in the development of typical 'reaction centers' can be found but they are far more numerous during the end of the second day after the injection of the bacterium.

It may be mentioned here that in unpublished experiments on the early reaction of the lymphatic tissue of the guinea pig to a minimum lethal dose of diphtheria toxin, the writer has observed this same sequence of activity in the lymphatic nodules. In the early stages of the reaction great numbers of new lymphatic nodules are formed in the same manner as described here. Only later do these nodules show the

necrotic changes typical for diphtheria. In these experiments with diphtheria toxin and with *B. monocytogenes*, as well as in experimental malaria in the monkey (Taliaferro and Cannon, '36, and Taliaferro and Mulligan, '37) the lymphatic nodule results primarily from an intensive lymphocytopoiesis in a given area and only later does it become a 'reaction center.'

The pliancy of the lymphatic tissue is further emphasized in the course of the infection with *B. monocytogenes* by the rapidity with which the architecture of the lymph node may change. At 36 hours after the injection, the cortex and the medulla are almost equal in proportion, but at 66 hours, the depletion of lymphocytes from the node is so extensive that nearly the entire node appears as the loose lymphatic tissue which is usually described as characteristic of medulla. The diffuse tissue of the cortex has almost completely disappeared. The demonstration of reticular fibers at this stage fails to disclose any 'nodular' arrangement of the fibers in the depleted area formerly occupied by 'cortex.' This labile arrangement of reticular fiber framework further stresses the interchangeability of the dense, the loose and the nodular forms of lymphatic tissue. Therefore, it is evident that we cannot draw any conclusions as to function and formation of nodules from any one stage of the process. The diffuse lymphatic tissue as well as the lymphatic nodule can undergo such marked changes in an exceedingly short time that one of these may turn into the other.

The lymphatic nodule thus has several functions. Primarily it may serve as a mechanism for the rapid production of lymphocytes whether the stimulus is physiological or pathological. In any series of normal animals a cycle of growth, maturation and regression in lymphatic nodules, as described by Maximow, can be found. His description, however, does not cover all of the aspects of the process. This lack resulted primarily from his failure to study experimentally controlled changes in the nodules. But on the other hand the denial of the lymphocytopoietic activity of the lymphatic nodules with claim for its sole function as a 'reaction center'

on the exclusive basis of human pathological material, is the building of a theory on singularly inadequate material. The tissues so obtained represent material collected haphazardly with respect to the duration of the process and the stimuli involved. But even greater than this deficiency is the fact that such material reflects the end stage of a disease process in which the resistance of the host has been so greatly depressed that death ensued. This same lack is to be found in those experimental studies which are based on a few widely separated intervals or on the termination of the process. Such material does not represent the lymphatic tissue and its nodules during physiological conditions or in those diseases and experiments in which recovery, not death, is the outcome.

In this study it has been demonstrated that the lymphatic nodules are primarily foci of proliferation of lymphocytes and only secondarily do they become 'reaction centers.' These new foci may arise at various times in any part of the lymphatic tissue; that is, they may appear anywhere in the lymphatic tissue of both cortex and medulla of the lymph node and the white pulp of the spleen as well as within pre-existing nodules in both situations. This explains the marked variations in the structure of the nodules. Hence the picture resulting from the appearance of a new focus of proliferation of lymphocytes within a pre-existing nodule depends on the state of the nodule at the time the focus develops. Thus, if the nodule consists of a solid mass of smaller lymphocytes in a reticular stroma, the new focus of proliferating lymphocytes will result in the appearance of a typical lymphatic nodule with a pale center in Flemming's sense. Further, if still another focus of lymphocyte proliferation develops slightly later within such a center of a nodule, the result is a lymphatic nodule with two secondary nodules of 'germinal center' type of different generations, which reflect different phases in the development and maturation of lymphocytes. In addition, at certain stages in various infections as those with *B. monocytogenes* here and as Taliaferro and co-workers have shown in malaria, it is not uncommon to find reaction

centers and lymphocytopoietic centers in the same lymphatic nodule.

A new focus of proliferation arising independently in the dense or loose lymphatic tissue may consist of medium lymphocytes and a few reticular cells. This area stands out sharply in the diffuse lymphatic tissue, appearing as a 'bare' germinal center which histologically is identical with that described by Flemming as occurring within nodules (plate 1, B).

CONCLUSIONS

1. In *B. monocytogenes* infection in rabbits and in guinea pigs the lymphatic nodule is primarily a focus of rapidly proliferating lymphocytes and only secondarily does it become a 'reaction center.'

2. The foci may develop independently of a pre-existing nodule.

3. New foci of proliferation may develop anywhere in the lymphatic tissue of the mesenteric lymph node or in the white pulp of the spleen.

4. Two or more cycles of proliferation may succeed each other in the same nodule.

5. In a previously developed nodule both lymphocytopoietic and reaction centers of different generations may occur at the same time, as well as two or more distinct areas of proliferation.

6. Each of the three types of lymphatic tissue—diffuse, loose or nodular—may turn into any one of the others.

7. The reticular fibers of the lymphatic nodules are not arranged in a permanent pattern but are mechanically pushed apart by the growth pressure of the lymphocytes.

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EXPLANATION OF PLATES 1 AND 2

Photomicrographs showing different stages in the cycle of the lymphatic nodule in diffuse lymphatic tissue of cortex of mesenteric lymph node of guinea pig, 5 days after injection of *B. monocytogenes*. $\times 450$, reduced by one-fourth. Hematoxylin-eosin-azure II.

PLATE 1

EXPLANATION OF FIGURES

A Small new nodule containing six mitotic lymphocytes. It is poorly demarcated from the surrounding diffuse lymphatic tissue and its margins are indicated by the arrow heads.

B Slightly later stage in the development of a lymphatic nodule, which is actively lymphocytopoietic. It contains twenty-seven mitoses in medium-sized lymphocytes. This nodule lacks completely, a peripheral zone of small lymphocytes such as is seen in plate 2, A. This nodule, histologically, is a typical 'germinal center' of Flemming.

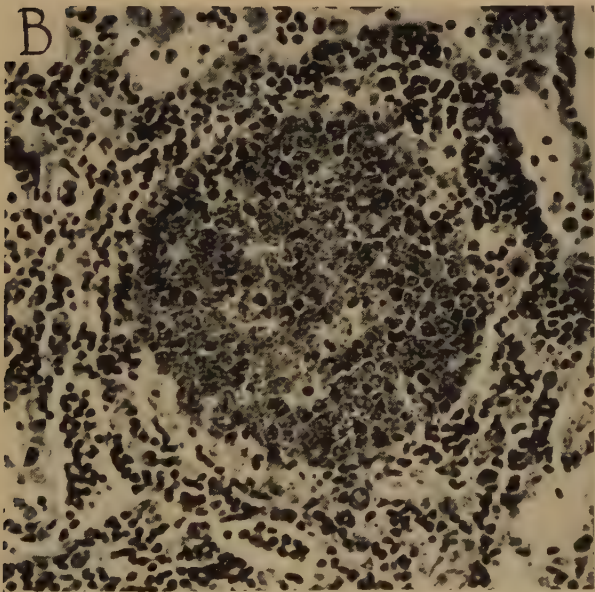
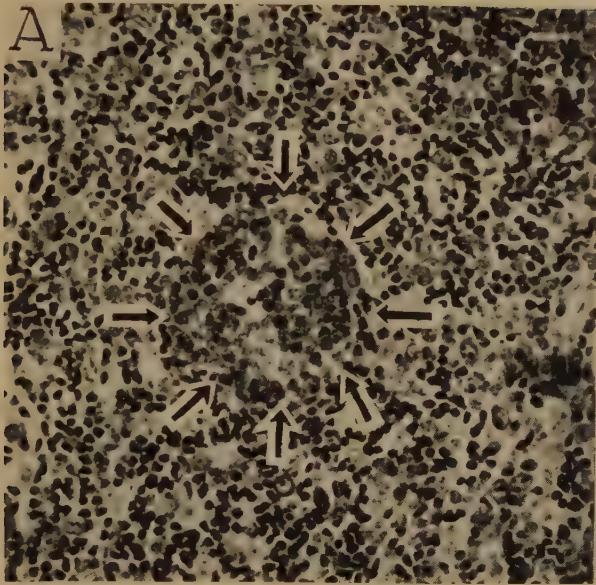


PLATE 2

EXPLANATION OF FIGURES

A The central portion of this nodule is still actively lymphocytopoietic and contains fifteen mitoses in medium-sized lymphocytes. It is demarcated from the surrounding diffuse lymphatic by more or less concentrically arranged layers of small lymphocytes. This is the type of nodule which is often regarded as typical, consisting of an outer dark-staining zone and a lighter central area.

B This nodule has a very pale-staining central portion consisting mainly of reticular cells, few macrophages and scattered smaller lymphocytes. The macrophages contain small amounts of cellular debris. The periphery of this nodule consists primarily of small lymphocytes which merge gradually into the surrounding diffuse lymphatic tissue. This nodule with its center depleted of lymphocytes is of the 'reaction center' type of Hellman.

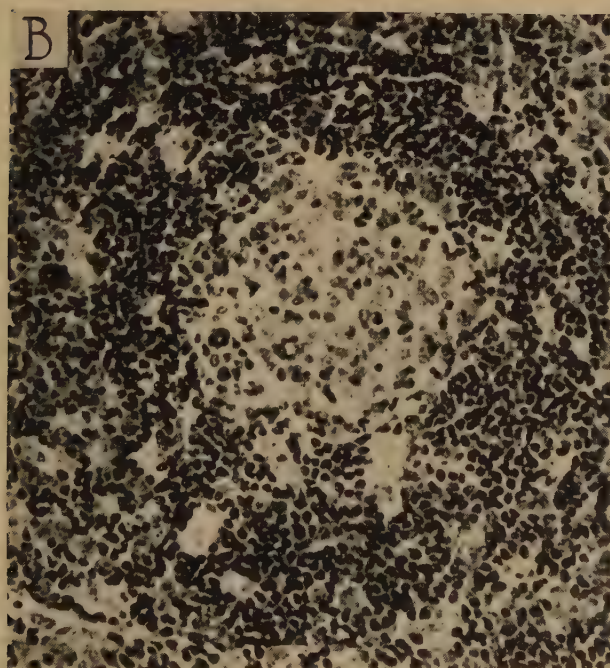
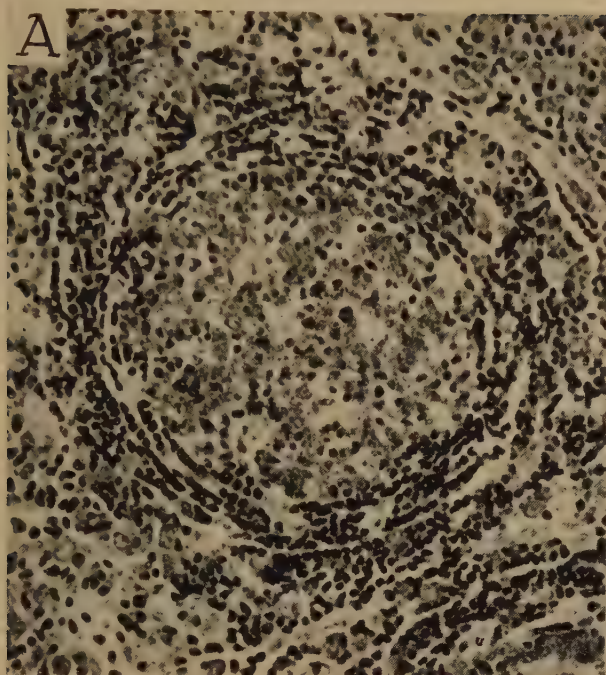
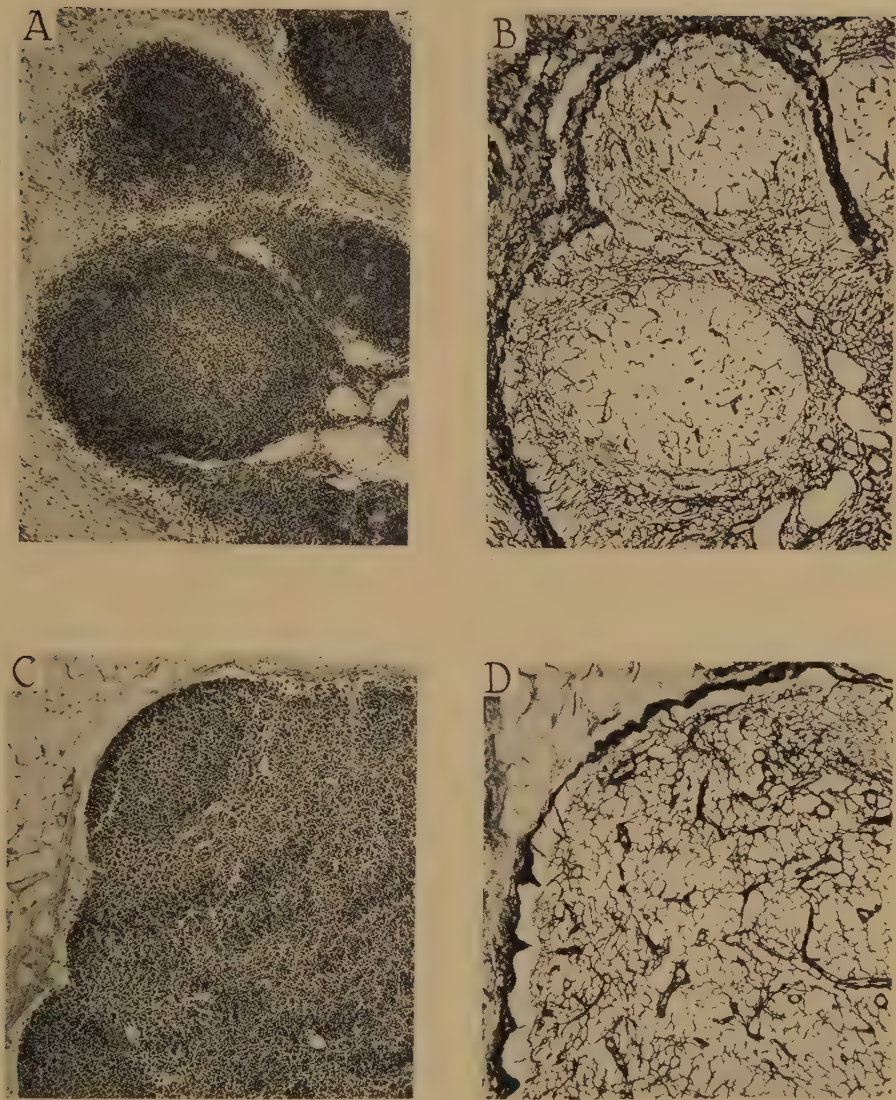


PLATE 3

EXPLANATION OF FIGURES

Figures A and B are photomicrographs from sections (not serial) of the same block of mesenteric lymph node of rabbit, 36 hours after injection of B. monocytogenes. Figures C and D from sections (not serial) of the same block of mesenteric lymph node of rabbit, 48 hours after injection of B. monocytogenes. These figures show that when nodular lymphatic tissue (A) becomes diffuse lymphatic tissue (C) the reticular-fiber framework, characteristic of the nodules (B), is lost (D). In all sections the subcapsular sinus is prominent and in A and B the extension of a trabecula into the lymphatic tissue is obvious. A and C stained with hematoxylin; B and D impregnated for reticular fibers by the Foot method. $\times 160$, reduced by one-half.



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